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Scientists in public

It is traditional for scientists to return to Europe from visits to the United States with their batteries recharged and a whole host of new ideas picked up in haste to be tried out at leisure. And it is equally traditional for those first few days after re-entry to be days of frustration, with limited funds, petty bureaucracy and inferior telephones. Gradually, however, there occurs a readjustment to the Old World and its range of values, almost like a shift of cultural Circadian rhythms. And the British can console themselves with the often-heard assertion that we may not have the greatest industrial vigour in the world, or a very significant growth in Gross National Product, but when it comes to the arts and sciences there are immense riches, and these are continually being fed by bright youngsters who, by some pleasing quirk of genetics or environment, seem to keep on coming in their droves.

Now much of this may well be true, although certainly as far as the sciences are concerned lack of industrial vigour is leading to a lack of new opportunities for young people to use their talents, which in a very short time could lead to young British scientific talent being famed mostly through its widespread presence in the laboratories of other countries. But one area in which the trans-Atlantic traveller certainly ought to notice that the United Kingdom is already painfully lacking in comparison with the United States is in the matter of the scientist and public life. It is not just that science has a more visible rôle to play in the United States, although this is undoubtedly true of a country with a vigorous space programme, a highly technologised military, serious energy problems, a commitment to the latest in telecommunications, microprocessors and so on. This inevitably means a wide, mature exposure for science in the media, with all the concomitant benefits. But it is also that the individual scientist is welcomed much more openly and equally into the discussion of public affairs and is expected not just to make specialist inputs appropriate to his or her discipline, but also to be the broad and balanced person that in Britain the arts graduate is expected to be.

This phenomenon not only occurs through the

existence of the in-and-outer in the higher ranks of public service in Washington—a category, incidentally, which will probably diminish as President Carter's campaign for purity on conflict-of-interest bites more deeply. It is to be seen also in the proliferation of quasi-governmental and private institutions around the nation devoted to public policy, which think it entirely natural to hire—and fire—scientists in numbers. It is certain that amongst them are going to be many who will just conform to the seedy wheeler-dealer image. But the system has thrown up and will continue to throw up many scientists who acquire a grasp of generalities and an overall profundity rarely to be seen in British circles.

Now many scientists on both sides of the Atlantic undeniably want, and should be granted the right to stay perfectly happily out of the public eye, getting on with their research. But as science and society cross each others paths more and more, society ought to be asking a growing number of scientists to abandon their specialisations, even temporarily, and to worry about broader issues.

We have, of course, in Britain an excellent and highly professional civil service, and it performs many functions admirably in the matters of science and public policy. But we pay a price for its very professionalism and that is a resistance to outside pressures. And with negligible movement into or out of the service either by administrators or 'professionals' above the age of about 30 there must be the most serious questions about whether the service doesn't waste genuine human resources. Here is a question which the Commons Select Committee on Science and Technology, if it were to be looking for some new and difficult ground to break, could well investigate. The role of the scientist in British society and the problem of too much compartmentalisation, which discriminates against the free flow of ideas, is a subject of broad interest. The results of an investigation would hardly be spectacular, nor given to immediate and drastic action. But long-term benefits could be substantial if the scientist who so wishes could be encouraged to lead a fuller life and to provide more in the way of public service. □



GDR's state of science

Scientific exchanges with the German Democratic Republic (GDR) are rare, and our knowledge of the state of its science is consequently sparse. A correspondent with some experience of East European countries, who recently completed a trip to several biochemistry and microbiology laboratories in the GDR, returned with these impressions.

THE mood among the scientists with whom I spoke is one of frustration and pessimism. The scientific community is thwarted and repressed by the government, and the situation is, if anything, worsening. I would put it as more critical than, say, in Poland or the Soviet Union. The very possibility of doing research in the GDR was a subject of discussion. The problems are of two sorts, financial and political.

One of my stops was a large city with a prestigious university. The contrast between the newly constructed university skyscraper, to be used primarily for the study of Marxism-Leninism, and the shabby buildings of the science complex made a striking impression. The biochemistry laboratory which I visited was not adequately equipped. The laboratory, with a staff of some 20 researchers, had, for example, no scintillation counter. In addition, some of the equipment which they did possess was of inferior quality. In particular, the ultracentrifuges, of East German manufacture, can be run for only a few hours at a time, or the drive will fail. Aggravating these financial problems is the peculiar bureaucratic regulation that purchases be made only once a year. This both wastes the meagre resources available to the laboratory—chemicals are ordered which are never used—but also leads to spot shortages which may abort an ongoing project.

Hard currency restrictions make subscriptions to Western scientific journals difficult to obtain. The biochemistry group had subscriptions neither to the *Journal of Biological Chemistry* nor to the *Journal of Molecular Biology*. The restrictions also greatly reduce the number of GDR scientists who can attend conferences in the West. At the time of my visit to another laboratory, for example, only one scientist out of some 800 total staff members was in the West. While science was a low priority area, the support of athletics was lavish. The new municipal stadium was monumental. Furthermore, much hard currency goes to support the hordes of javelin throwers, swimmers and runners who are treated to expensive trips to Western Europe and North America.

The political situation complements the economic one. The government plays a very direct role in laboratory affairs. Needless to say, permission is needed to attend a scientific conference outside the GDR, but is also needed, for example, to publish an article in a Western scientific journal. The government can and does direct a laboratory programme; a microbiology laboratory on my itinerary had been ordered to abandon its programme of basic research and to commence work on a project which the administrators felt had industrial applications. Even in basic research, 'plans' are delivered to the researchers detailing experimental 'goals'. With the exception of the Communist Party members of the staff, these 'plans' are largely ignored. Recently, however, it was decided that the laboratories must hire more Party members. For one group, this meant that all future appointments at the level of Laboratory Chief or above would have to be filled by Party men.

The authorities have also found that science administration is a good place to put retired military personnel. In

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some cases these ex-colonels have chosen to classify a laboratory as 'secret', shutting it off from communication with the rest of the scientific community.

Pressure is applied on the scientists to join the Communist Party, but it is my impression that this is far from successful. A valid reason for not joining is the profession of a religious belief, and this excuse is used frequently. Although promotions, trips abroad and other rewards are given preferentially to Party men, these inducements are not sufficient to overcome the moral repugnance which most of my informants felt towards Party membership. Other scientists, less estranged from Communist ideology, spoke of "compromises" that have to be made in order to work effectively.

In spite of these difficulties, science has not been abandoned; research of good quality is still going on. Efforts to keep up with scientific progress are evident. Especially apparent is the enthusiasm with which Western visitors are greeted; the GDR scientists clearly want to establish contacts and exchange ideas with their Western counterparts. If we wish to aid them, we must visit their laboratories and attend meetings in the GDR, since our Eastern colleagues cannot easily leave their country, or initiate contacts with Western scientists. The exchange of scientific information, as well as gifts of subscriptions to journals or of chemicals and laboratory supplies could help science survive. □

International science (1)

IMS at the crossroads

The success of next week's ISEE satellite mission is crucial to the IMS. **Judy Redfearn** outlines why

PART of the International Sun-Earth Explorer (ISEE), the second major satellite mission in the International Magnetospheric Study (IMS), was due for launch this week aboard one of NASA's Delta launch vehicles. But because of delays to NASA's launch programme caused by the failure last month of the Delta rocket carrying the European Space Agency's (ESA) Orbital Test Satellite (OTS), the ISEE launch has been put back, probably until 19 October. If the launch fails to go ahead then, or at least by the end of the month, it will face a lengthy hold-up because the current period of short daily launch windows will then have passed by; the ISEE mission would have to remain earthbound until next spring.

Magnetospheric scientists will be watching the launch with a degree of apprehension for another reason. Earlier this year, a Delta launcher failed to send Europe's Geos, the first IMS satellite mission, into geostationary orbit. Although the consequences are not as severe as was first thought, the IMS programme has suffered a setback. So the success of ISEE is now more important than ever to the success of the IMS. If all goes well next week, a unique programme will begin to find out the precise mechanisms governing the magnetosphere, the area of the near-earth environment where the solar wind interacts with the earth's magnetic field.

For the first time two satellites, NASA's ISEE A and ESA's ISEE B, are to be launched in tandem aboard a Delta 2914 rocket into the same highly elliptical orbit. ISEE B, the 'daughter', will follow ISEE A, the 'mother', at a distance varying between 100 km and 5,000 km. The satellites will carry carefully matched experiments allowing them both to make comparable measurements of the same phenomena. Because they will be moving at a known speed and distance apart, they will be able to distinguish between transient phenomena and those which are permanent but vary in space. A single satellite suffers from not knowing whether a phenomenon has moved past it or has died out.

Crossing boundaries

Although many phenomena are known to exist in the magnetosphere, they are not well understood because of the problem of distinguishing spatial from

temporal variations. In particular little is known about the processes which occur at the boundary where the solar wind meets the magnetosphere. The highly elliptical orbit of ISEE A and B was designed to maximise the number of crossings of this boundary, or bow-shock, during the satellites' three-year lifetime. The orbit chosen has a 59-hour period with a closest distance from earth of 280 km and a furthest distance of about 140,000 km. It will also carry the satellites through other boundaries, such as the magnetopause, plasmopause and neutral current sheet.

In June next year, ISEE A and B will be joined by the third and final part of the ISEE mission, ISEE C. Unlike A and B, C will be launched into an orbit around the sun at a distance of about 1.6 million km from the earth. For fuel economy, the orbit was chosen to lie on a libration point where the gravitational forces between the sun, earth and moon almost cancel out.

While A and B are measuring processes in the magnetosphere, from its position in deep space C will be monitoring variations in the solar wind and sending information on solar particles back to earth an hour in advance of the particles reaching earth themselves. The data will give a picture of the solar wind which will help in understanding observations made by the A and B satellites.

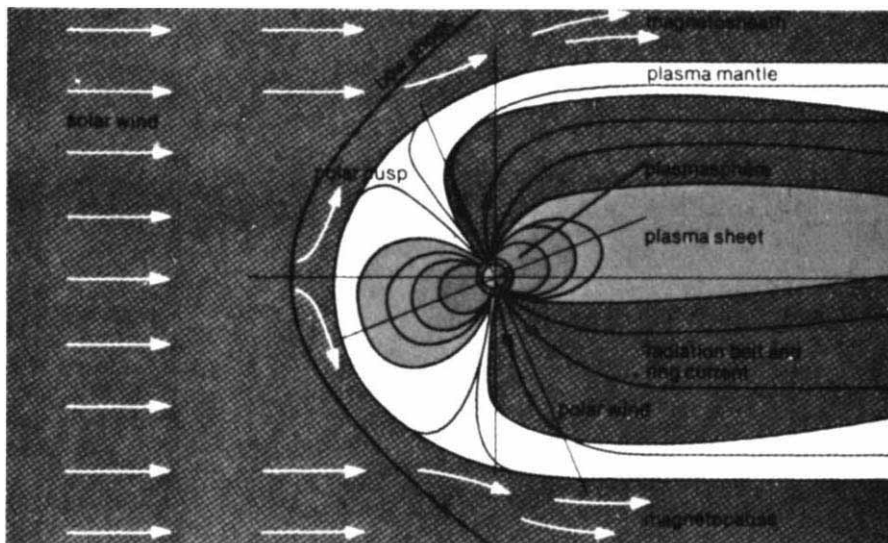
At the end of the mission, scientists should know a lot more about the solar wind and the processes governing the magnetosphere, which may in turn lead to a better understanding of radio

transmission on earth and some of the mechanisms effecting climate. The magnetosphere, a natural plasma laboratory surrounding the earth, is like the earth's own small universe, the solar wind taking the part of the hot plasma which makes up most of the matter in space and the earth's magnetic field taking the part of the magnetic fields found in space. The study of how ionised particles interact with the earth's magnetic field should yield much about the fundamental processes which govern the behaviour of cosmological objects such as radiogalaxies, pulsars and radio and X-ray stars.

Setting up the IMS

By the late 1960s scientists had a good picture of the magnetosphere, but they knew little of the dynamic processes controlling it. The International Magnetospheric Study was set up as a co-ordinated attack on the remaining problems. To organise the study, the International Council of Scientific Unions (ICSU) Special Committee on Solar-Terrestrial Physics set up an IMS steering committee with representatives from various scientific unions, COSPAR and satellite launching agencies. Thirty-nine countries are now taking part in the IMS, which began in 1976 and is due to run until December 1979.

It is the first international programme of its kind to be based on spacecraft flown by several different agencies. Fourteen spacecraft launched during the IMS period are planned to carry experiments directly relevant to the study and 25 launched before 1976 are expected to add valuable data. But at the centre of the IMS are two spacecraft missions devoted entirely to magnetospheric science—Geos and the ISEE. In addition, Japan is contributing with its magnetospheric satellite, Exos, and Russia with a near-earth satellite.



Cross-section of magnetospheric plasma distribution

The spacecraft programme, however, would be of little value without the numerous ground-based rocket and balloon projects which will continue throughout the IMS period. Earth-based measurements of variations in the magnetic field can be used to monitor magnetic storms and plasma waves and even to predict changes in the magnetosphere. Correlating these measurements with satellite observations should indicate how changes in field strength affect the shape and dynamics of the magnetosphere. Many of the 39 countries which could not afford to be involved in the space programme are contributing to the IMS through a sophisticated world-wide network of ground observatories. Even at the beginning of 1976, more than 1,000 individual research projects were registered on the IMS computerised file.

Many different experimental programmes working towards the same end thus need coordinating. Occasionally, for example, two or more satellites may be in such a configuration that

simultaneous observations would be very valuable. Similarly, satellite observations should sometimes be timed in with ground-based or rocket experiments. To coordinate observations, NASA helped set up an IMS Satellite Situation Centre at the beginning of the study at the World Data Center A for Rockets and Satellites at the Goddard Space Flight Center, Boulder, Colorado. Its function is to report on satellite positions, recommend periods of special interest, compile and up-date information on satellite experiments and answer inquiries on special projects, experiments and the whereabouts of satellites. Every month, the IMS Central Information Exchange Office, also at Boulder, produces an IMS news letter.

Regaining ground

Hopes for regaining the ground lost by the partial failure of Geos hang on the possibility of launching the second Geos satellite into geostationary orbit early next year. The intention had been to place the first Geos in a geo-

stationary orbit 36,000 km above the equator along a field line connecting the north and south auroral zones so that particles on their way to the aurora would pass through it. Ground-based experiments and rocket-borne observations of the aurora could then have been coordinated with experiments on board Geos.

But Geos is not in a geostationary orbit and coordinating observations is very difficult. It would have been impossible had the Geos ground control at Darmstadt not managed to manoeuvre the satellite into a doubly geosynchronous orbit shortly after the unsuccessful launch. Geos now passes through the neighbourhood of the planned geostationary orbit twice a day, meaning that coordinated observations are possible, but only for a very short time. If Geos 2 were launched next year, it could take the place of Geos 1. It would have to be launched on another Delta rocket, however, and ESA might decide to wait until the end of 1979, when it could be launched on Europe's launcher, Ariane. □

International science (2)

WHO's show on the road

Peter Collins describes the gestation of the World Health Organisation's tropical diseases programme

THE temptation to launch global campaigns against specific diseases or conditions of mankind is one the World Health Organisation (WHO) seems unable to resist. The most recent and from all appearances most successful is the smallpox eradication campaign. Pockets of the disease remain, principally in the horn of Africa, but the campaign's objectives are in sight in under ten years. By contrast, in spite of the malaria eradication campaign, which started in 1955, malaria has never been anywhere near eradication in much of Africa south of the Sahara, and is now increasing in other regions and countries where eradication was thought to have been achieved. Now WHO's thinking has turned towards a more broadly-based approach. It is well exemplified by the Special Programme on Research and Training in Tropical Diseases which is tackling six diseases (malaria, schistosomiasis, the filariases, trypanosomiasis, Leishmaniasis and leprosy) that affect some 700 million men, women and children.

Perhaps the greatest single constraint on the development of the tropics lies in the burden of disease borne by their human inhabitants. Probably one in every four people alive

today is at risk from one or more of the six diseases with which the programme is concerned. The position worsens every year as population increases and the world's pharmaceutical industry stands back. No new drug against trypanosomiasis has appeared in the past 20 years; the only drugs available against the filariases have side effects so serious that their use for mass treatment is out of the question; and the drugs used for treating malaria are meeting with more and more resistance on the part of the parasites. Added to this is the increasing resistance of many of the vectors of these diseases to the chemicals used against them, and the many reservations about the use of DDT, still often the most effective weapon for insect vector control.

The sheer lack of scientific knowledge about many of these diseases is also a problem. Not enough is known about the biology of the parasite and the mechanisms by which it eludes the defences of the human host to allow intelligent intervention to be planned. But great advances in such fields as immunology have led to the evolution of a whole armoury of new weapons for the prevention, diagnosis and treatment of disease afflicting richer



WHO anti-malaria emblem

countries, and the question is how these can be made available or adapted to help control the great endemic diseases of the tropical countries.

WHO's answer

The present programme is WHO's answer to this question, but it originated within WHO from the conjunction of two quite separate ideas. In 1964 an expert group of immunologists and parasitologists was assembled for the first time in Geneva. Little common ground seemed to exist between these two disciplines thereto: the parasitologists considered that there was little if any immune response to the diseases with which they were concerned, and the immunologists had found ample scope, backed by adequate funds, in fields like cancer research. After this meeting, however, things began to change, and while the immunology of

parasitic diseases was being examined, another initiative was being taken within WHO—the establishment in certain African countries of a network of biomedical research centres, eventually to be staffed and run by local personnel. The feeling was that only research by nationals of the countries concerned could achieve real control of tropical diseases.

It was from a 'meeting of minds' among senior staff involved in these two developments that the present programme evolved. By 1974 their ideas were ready for presentation to the World Health Assembly and subsequent examination by WHO's Advisory Committee on Medical Research (ACMR). The basic plan, further discussed with prospective participants, was developed in time for a major 'housekeeping' meeting in December last year. The pattern is that of a co-operative effort of donor and recipient governments, international agencies and specialised institutions, collaborating with and coordinated by WHO. A major part as co-sponsors will be played by the United Nations Development Programme (UNDP) and (though its precise rôle has yet to be determined) the World Bank. Because the programme involves a number of different WHO departments, a small Tropical Diseases Research Group (TDR) has been set up for overall scientific coordination and management. Progress will be reviewed annually by a Scientific and Technical Advisory Committee composed of scientists, selected on an individual basis, to make the best existing knowledge and experience available to those directing the programme. General oversight will be ensured by a Joint Consultative Board, expected to be convened later this year and designed to represent all the various groups of participants in the programme.

The programme is expected to cost between \$10 and \$20 million a year as presently foreseen. This money will come from governments through their development aid or technical assistance allocations, and from other sources of international funding. It is here that WHO might have anticipated some difficulty, for those concerned with development financing are naturally somewhat cautious when asked to support what is, admittedly, a vast experiment, at a time when funds for development projects are increasingly tight. Several different approaches to this type of financing are already apparent. The Scandinavians prefer to hand over funds directly, often seeming to take little interest in their management, although they may now be looking harder at the results than hitherto. The USA, on the other hand,

appears to favour complete control of the programme funds, preferably by the World Bank. Britain's fairly neutral stand on that point is tempered by an insistence on continuing and critical evaluation of both the selection and progress of individual projects. Even at this early stage, several governments, Britain's included, have been highly critical of the potential of certain projects to which programme funds have been allocated by WHO. Intriguingly, it does seem that one problem is to find sufficient projects to absorb the funds already available, a situation that should reassure those who fear that the programme will prove to be yet another drain down which development funds are being uselessly poured.

Two lines

The two main lines of action that make up the agreed programme reflect its dual origin within WHO. The first is a vastly increased research effort directed at some of the main problems of the six selected diseases, and involving many of the ideas and techniques developed by biomedical research into the diseases of the industrialised, affluent world. Where possible, this research should be done where the diseases occur, and eventually (though this is not yet feasible) by nationals of the countries concerned. The second recognises that research, however successful, is of little use unless it can be properly exploited. This means training nationals of the tropical countries in the design, administration and application down to village level of whatever control system is finally evolved.

The underlying approach is not without precedent as far as WHO is concerned. Its programme for research into human reproduction was set up on much the same lines: a group of scientists was assembled, presented with certain ideas, asked if they thought a programme based on these ideas could be made to work, and urged to indicate the steps to be taken. This in itself was something rather new; biologists are not usually asked to do research with a definite end in view, and the human reproduction programme is one of WHO's current successes. That the system can work is also shown by the Scientific Working Group on the immunology of leprosy, which was set up in the same way and is already yielding results.

The parasitic diseases programme is of a different order of magnitude, though. Each of the scientific working groups is in itself an experiment directed to separate objectives. Apart from those dealing with leprosy, groups on filariasis, schistosomiasis and the immunology of malaria have been set up; others will be concerned with

such broad fields as vector biology and the tissue culture of parasites. Each group examines its particular disease under headings like immunology, chemotherapy, epidemiology and training, and identifies desirable research projects and the institutes and individuals to tackle them. WHO envisages a network with a coordinating group at headquarters. On the one side are the 'collaborating institutes', the established centres of biomedical research, mostly located in the industrialised countries. Their collaboration will be sought for the more sophisticated research especially where new and special equipment and facilities are needed, and for training at the higher levels. On the other side are 'collaborating centres', at various levels from large university departments to small laboratories employing only a handful of qualified staff, and located mostly where the diseases are prevalent. It is in this group that the 'institution strengthening and training activities' of the programme will have first priority.

No cutting

As WHO sees it, there is no need for the programme to cut across the activities of the established institutions in the industrialised countries. But with too many people doing research into tropical diseases who have had little or no contact with those suffering from them, a properly coordinated operation of such a network should help progress. WHO would make a further point, that the programme offers opportunities that are, scientifically speaking, exciting, with the use of the new tools and high technology of modern biological science. The different approach to publicising the programme reflects this. In the past, publicity has been given to global programmes of public concern, with the aim of telling people about something being done for their welfare—the conquest or alleviation of rheumatism, for example (1977 is World Rheumatism Year). For the tropical diseases programme, WHO's aim is rather to interest the scientific community.

With the gradual run-down of the great colonial medical services, tropical disease research has been increasingly less popular. But now, the more people who know of this vast new programme and of its funding, the greater likelihood there is of interesting young research workers in the opportunities it has to offer. And at some \$20 million a year for, say, 20 years, it is not that expensive. A major pharmaceutical company can spend five times that annual sum in a single year, and some countries spend as much on cancer research as the total budget for this research on tropical diseases. □

BRITAIN

Blunt then blunted

The UK Medical Research Council (MRC) published its latest annual report earlier this week. Chris Sherwell reports

APART from being mere snapshots of the period they describe, annual reports suffer by emerging halfway through the following year. Introducing the first report since he became MRC Secretary, James Gowans quickly addressed one problem by communicating a vivid impression of the dynamic quality that informs current medical research. But the need to bring everyone up to date blunted the impact of the report's potent, if measured, criticisms of the way some of that research is now run.

The report, which is for the twelve months to March, describes the impact of a £1 million (10%) cut in commissioned research from the Department of Health and Social Security (DHSS). It says the Council was "deeply concerned" at the size and suddenness of the reduction, which "disrupts financial planning and frustrates initiatives" and affects both commissioned and other research. But the outlook was now less bleak, Gowans told his audience. The DHSS would be giving back £700,000 next year, and the MRC "firmly expected" a 1.6% real increase in its share of the Science Budget in the coming year.

Gowans was apparently concerned also to moderate the tone of the report's criticisms of the operation of the customer-contractor principle, under which the DHSS acquired control of one-fifth of the MRC's funds. A series of rhetorical questions in the report suggests that responsibility for any lack of orientation of research work to social objectives cannot be laid at the MRC's door. The report also describes at length how administrative work and accountability requirements have imposed heavy burdens and taken experts away from research—"a high price to pay" for arrangements which could be achieved "with less

formality and administrative expense".

Gowans amplified the first of these points and dwelt not at all on the second. He described specific cases—the latest being the Blood Group Reference Lab—of the MRC deciding to start something new and then handing it on to an enthusiastic government; the MRC, he said, was anticipating national needs long before the customer-contractor principle came along. The ease with which the MRC programme merged with DHSS requirements, he added, also vindicated the MRC's judgment; the DHSS wanted the MRC to do what it was doing anyway. But the conclusion he drew was that DHSS-MRC relations were very close. The "one proviso" was a complaint about bureaucracy.

If these pulled punches reflect the happy amelioration of financial problems, other thrusts in the report indicate that additional anxieties remain. The organisation of clinical research is "becoming increasingly difficult" as the incentives for young doctors to do research have diminished, and there is an "apparent tendency to lose sight of research as a proper component of clinical training". An appropriate pattern of support for university research when universities are not expanding has also yet to emerge, and is "of crucial importance" if the flow of able young scientists into research is to continue; holding research teams of high quality together will be "increasingly difficult" unless the problem of low numbers being absorbed into permanent university appointments is solved.

In the stringent economic circumstances the Council describes its first priority as reducing long-term commitments in order to free money for new work. Competition for long-term support will thus be fierce, it says, but short-term awards will now increase, and it urges the scientific community to continue to apply for these.

On the subject of recombinant DNA experiments, the MRC says it is looking forward to receiving proposals (four

large project grants have already been made to universities); "high priority" should be given to the provision of centralised facilities for category III and IV experiments. The MRC in fact has no plans to build a category IV facility. The future of the only one in the country, the Microbiological Research Establishment at Porton, is now under consideration by the government. An MRC committee set up to examine the possibilities it offers is due to report soon. □

Research report

THE massive UK Department of the Environment (DOE) last week released a slim fourth annual report on research and development—covering a period that ended a full 18 months ago. A press release accompanying the report, which deals with the last full year before the department was divided into the Departments of Environment and Transport and before Martin Holdgate succeeded Dennis Lyons as Director General of Research, does not explain the delay. But it does advise, without amplification, that recent organisational changes under Holdgate will be described in the 1976-77 report. Holdgate is also expected to outline them to the press next week.

In the meantime, some of the details are available in the September issue of *Environment and Transport World*, the newspaper of the two departments. They are not exactly minor but, unsurprisingly perhaps, a couple are apparently mere name changes; thus, the Directorate of Research Requirements has become the Directorate of Research Policy, and the Research Secretariat has become the Research Administration Division. The Systems Analysis Research Unit (SARU), on the other hand, which has been looking at global problems (see page 356), has not changed its name but is, according to the paper, "increasingly turning its attention to problems on the domestic front".

Perhaps the most important change is the reduction from fifteen to six in the number of internal Research Requirements Committees. These will consist of what Holdgate calls "triads", three-man teams with a representative from the policy side who want particular work done ('customers'), from department research establishments or other research centres ('contractors'), and from the central research policy division. The six committees embrace areas controlled by senior civil servants like planning, countryside and resources, transport, construction and environmental protection.

As for last week's report, it says expenditure on research (at a peak of some £45 million for 1976-77, of which about a quarter went on environmental pollution and resources) is expected to decline sharply to a level in 1978-79 close to that five years ago. This, it says, is a result of constraints on public expenditure and reductions in civil service manpower.

Chris Sherwell

Five-year pattern of MRC expenditure

	Total Expenditure (£million) ¹	Change % ²	Real change % ³
1972-73	28.510 (1.737)	+14.3	+4.1
1973-74	30.330 (5.765)	+6.4	+2.5
1974-75	36.293 (8.745)	+19.7	-1.3
1975-76	47.140 (13.148)	+29.9	-0.9
1976-77	52.166 (13.315)	+10.7	+0.9

¹ Figures in brackets are income within the total from sources other than the MRC's allocation from the Science Budget (eg. government departments, health authorities)

² Change in total expenditure over previous year (undeflated)

³ Change, in real terms, of MRC allocation from the Science Budget, over previous year

USSR

Exchange agreement

In spite of new agreements, there is scope for more scientific visits between the UK and USSR. Vera Rich reports

A NEW agreement on the exchange of scientific visits was signed in Moscow last month by Lord Todd on behalf of the Royal Society and Academician Anatolii A. Aleksandrov for the Soviet Academy of Sciences. Although the negotiations took place in what Aleksandrov described as a "friendly and constructive" atmosphere, and although Lord Todd told *Nature* that he felt that "good progress had been made", the details of the latest agreements reveal just how much progress in scientific exchange still remains to be made.

The new agreement provides for a two-way traffic of seven senior and four junior visits per year. Senior scientists will visit the host country for 3–6 weeks and junior scientists for 8–10 months at a time, the total yearly

quota being 40 man-months. This seems negligible compared with the 50–100 short-term visits per year between the UK and the major EEC countries or with UK–Poland exchanges, where there is an annual flow of some 80 visits to Britain and 40 visits to Poland. Leaving aside the special case of the English-speaking countries, one would expect the flow of exchange visits to be roughly proportional to the size and scientific importance of the country concerned. The anomalous position of Soviet–UK exchanges is apparently recognised by both parties; according to Lord Todd, both sides agreed that in so far as finances permit, they would like to see the number of exchanges increased, especially among junior scientists.

There is no great enthusiasm in the UK, however, to take up even the number of exchange places available. One reason is obviously language; most Soviet scientists have at least a reading knowledge of English, but the need for a reciprocal knowledge of Russian has

largely been obviated in recent years by the expansion of cover-to-cover journal translation programmes. It is also extremely difficult for young British scientists to discover how they would benefit from an exchange visit.

The Royal Society delegation pressed for more explicit information on the work being done at the various Soviet Institutes. They were assured that intending visitors could find all they needed to know from journals such as *Priroda* (Nature) and *Nauka i Zhizn'* (Science and Life). These semi-popular journals are, in fact, designed to give a general idea of current progress rather than to specify the work of individual scientists or institutes; moreover, they are in Russian (the translation programmes deal with specialist journals only), as is the more informative *Vestnik* (Bulletin) of the Academy of Sciences.

The new agreement does, however, include one rather promising provision—the extension of joint research projects. Working visits between the teams involved would not necessarily come out of the 40 man-months of the basic programme. □

USA

Rock energy

David Davies reports from Washington on the US programme to exploit geothermal energy

WITH the United States importing nearly half of its petroleum now, it is no wonder that even modest contributions to the nation's energy budget assume a growing importance. The geothermal energy programme in the US Department of Energy (DOE) could only displace a few per cent. of the nation's fossil fuel requirements by the year 2000 but is authorised this financial year to spend \$116 million, double last year's authorisation and nearly five times the figure of four years ago.

Resources are divided into three categories of increasing magnitude but also of increasing difficulty of exploitation. Convective hydrothermal resources comprise both dry-steam systems, of which the Geysers field in California (extracting 500 MWe at present but eventually 2,000 MWe) is probably the only US example, and liquid-dominated reservoirs, of which there are many at temperatures upward of 90°C. Geopressured resources consist of methane dissolved in water at

moderate temperatures. The water is in isolated reservoirs at the pressure of the overlying rock, much greater than the hydrothermal pressure, and heat, methane and hydraulic power can all be extracted. The resource is concentrated around the Gulf states. Finally there are 'hot dry rocks', rocks at relatively high temperatures near the surface into which water must be introduced to extract the heat.

Dry steam already provides 5% of California's electrical needs at a very attractive price and has needed little government support. Liquid-dominated reservoirs, on the other hand, have needed a stimulus; DOE has just issued a request for bids to build a 50 MWe demonstration plant in the western United States that will be economic and socially and environmentally acceptable. The department hopes to sign a contract for this in mid-1978. But the real and growing optimism, according to Dr James Bresee, director of the programme, is in the potential of the hydrothermal resource for direct-heat applications.

Geopressured resources have a much larger question mark against them. There are major unanswered issues of reservoir producibility and longevity, and so far there has been little com-

mercial interest. Nevertheless, DOE plans to go ahead this year with well-tests in Texas and Louisiana, and has proposed pilot plants for energy recovery.

The hot-dry-rock experiment at Los Alamos, although it has attracted the widest attention, must at present be regarded as the most speculative of all. A fractured zone has been created at a depth of 3 km in granite, and water is run down one pipe, through the granite, and up through a second. The rising hot water flashes to steam. Less than 10% of the water is lost underground. So far the heat extracted (about 3 MWt) is less than had been expected, so the next few months will be a crucial time for this programme.

Not all the obstacles to geothermal energy are technical. DOE employs about six professionals simply to look at the so-called institutional barriers—tax laws, environmental regulations and so on. At last count at least six governmental departments and over ten major agencies are involved in some way or another. Further, although the federal government has established loan guarantees for up to 75% of any loans to the geothermal industry, the widely-expected flood of applicants has not materialised; so far only two guarantees have been made, apparently because large companies would rather act as their own guarantor. □

CANADA

Add minister

The Canadian cabinet has a new science minister. David Spurgeon reports from Ottawa

CANADIAN scientists are once again feeling hard done by as a result of a federal government cabinet shuffle. Hugh Faulkner, Minister of State for Science and Technology since 1976, has been shifted to the portfolio for Indian and Northern Affairs, and a new science minister, J. Judd Buchanan, appointed in his place. He is the fifth minister to have been appointed since the ministry was established about six years ago.

Grumbling was also heard when Faulkner was appointed just two years ago. But in his short time at the post he earned the respect of many in the scientific community by his obviously sincere concern, his interest in the field and his desire to learn. He did not come to the ministry with what many scientists felt was the ideal background. He nevertheless took time to listen, and many scientists feel they have now lost an ally if not a friend. All Faulkner's homework will now go for naught, and Buchanan will have to start over again.

The shift is still more galling for the scientists inasmuch as it indicates once again that the federal government does not take science seriously. For apart from Buchanan's own lack of familiarity with the field, the task becomes once more a part-time concern of its minister. When C. M. Drury held the portfolio, he also held that of Public Works.

Buchanan will now revert to that arrangement, but he'll have even less time than Drury to think about science because his job has been as a sort of 'lieutenant' for the Liberal party in Ontario. Buchanan went from the ministry of Indian and Northern Affairs to the less onerous Public Works portfolio before coming to Science and Technology in order to have time for these political duties. Now some are wondering how he is going to deal effectively with all three areas.

Some very senior scientists, indeed, are beginning to question the usefulness of a system that depends on firm and effective communications lines being established between a minister and the scientific community, and then breaks these lines with impunity just when they have become established. Perhaps the new minister will be able to prove they are wrong, and that such a system can work well after all. It is a heavy responsibility. □

WEST GERMANY

Solar sell

Werner Gries reports from Bonn on state support for energy research

WEST GERMANY'S support for energy research developed a stage further last month with the announcement from the federal Minister of Research and Technology, Hans Matthöffer, of a large programme of support in the field of solar energy. The federal government has supported research into direct and indirect utilisation of solar energy since 1974, and the new programme, *Technologies for the Utilisation of Solar Energy*, makes available a total of DM166 million over the four years 1977-80 to supplement expenditure by private industry.

Some 76% of the country's total energy requirement goes on heating, and 24% for lighting and power. Space heating alone absorbs 40%, mainly through burning mineral oil. Solar energy is therefore important because it can be used for such low-temperature purposes as hot water supply and heating in residential buildings; but its low intensity and its variability mean an efficient technology is needed. Solar radiation per square metre in Germany is only half that in the USA.

The government's measures to promote solar energy hitherto have extended to 50 projects, requiring a total contribution of DM50 million of government money. Chief of these have been the development and long-term testing of novel types of solar collectors. At the same time mass production processes for solar collectors are being developed and a few demonstration plants both in public installations as well as in the private sector have been built with government funds.

Results so far show that it is technically possible even in West Germany to satisfy fully the heating requirements of a well insulated single-family house with solar energy. The necessary systems and appliances are already being offered on the market but the financial outlay is so high that it does not pay to put them in at present. Therefore, in addition to their R&D measures, the government introduced at the beginning of September a programme to encourage energy-saving investments. This grants allowances of 20% of installation costs for insulation, heat pumps and solar appliances.

The conversion of solar energy for purposes other than low-temperature heating has lower priority, but the federal government is promoting R&D in electricity generation to open up new export prospects for German in-

dustry in countries which have plenty of sun. Solar collectors for hot water supply will receive support in the future. Private investment will be rewarded by allowances, model public building measures will explain the new techniques, and standardisation of the most favourable appliances will have an important place in government promotion measures. But the extension of solar installations for space heating depends on the development of energy storage systems, and R&D in this area is being intensified. Solar engineering systems for heat production up to 200 °C are another important focus of research, and the government is making funds available for demonstration plants to show their practical application.

In another trench on the energy front, meanwhile, discussion of the advantages and disadvantages of breeder reactors and the risks attached to them is becoming more heated, notwithstanding the recent unexpectedly quiescent demonstration at Kalkar, the site for a 300 MW prototype breeder. A recent court judgment called into question the construction of the reactor when the judges requested the Federal Constitutional Court to inquire into whether the building of breeder reactors is consistent with the law as it stands at present.

Arguments have persisted in parliament. The Minister of Research and Technology is responsible for financing the development of breeder reactors, and he has given the Bundestag a detailed account in support of breeder development. A total of DM3,000 million has been distributed by the state for research work in this field, annual expenditure is running at DM470 million, and 8,000 people are employed in research, development and construction. Development is marked out up to 1982 with the prototype at Kalkar and the Franco-German *Superphénix* in France.

Though the idea is being aired of severely reducing breeder reactor development to no more than a minimum, the minister has come to the conclusion that cutting the building work on the prototype would cost just as much as terminating it. Many contracts have still to be fulfilled and high social costs would accrue in paying off workers and paying the stipulated penalties for the co-partners in Belgium and the Netherlands. The government maintains that plutonium presents no unjustified risks, contending that the technology can be mastered and that the potential danger is no greater than with a lot of other materials. □

IN BRIEF

USSR space set-back

The cancellation this week of the *Soyuz 25/Salyut 6* link-up is a serious set-back to the Soviet space-programme and a blow to its prestige, especially as some major exploit to mark the 60th anniversary of the October Revolution was widely expected. It also stresses, once again, the great reliance placed by Soviet planners on automatic control even of manned spacecraft. According to the Tass announcement, the two craft were automatically brought close together, and then a docking operation was carried out from 20 metres. "Deviations from the planned docking regime" led to the decision to abort the mission.

Antarctic meeting

The main achievement of the Antarctic Treaty meeting, which ended in London last weekend, was to agree on interim arrangements for conserving marine living resources. Details were not immediately available but the drift of the discussions has been clear.

First the agreement is voluntary. Second it looks at this stage as if it will be confined to restraints on catch quotas of krill and the exchange of statistics. The agreement can only be temporary because West Germany, which has been active in the field, is not a signatory of the Treaty.

The Treaty powers have also agreed to extend their voluntary moratorium on minerals exploration and exploita-

tion. Experts attending the meeting think that it will be at least five years before enough scientific data has been accumulated to assess the likely environmental effects of any exploration and exploitation.

Telescope agreements

ESA is to contribute a faint-object camera and a solar array to NASA's Space Telescope programme under an agreement signed last weekend. The telescope is due for launch in 1983.

Progress has also been made in the Dutch-American Infra-red Astronomical Satellite (IRAS) project with the award last week of a contract for construction of the satellite by the NIVR in Holland.

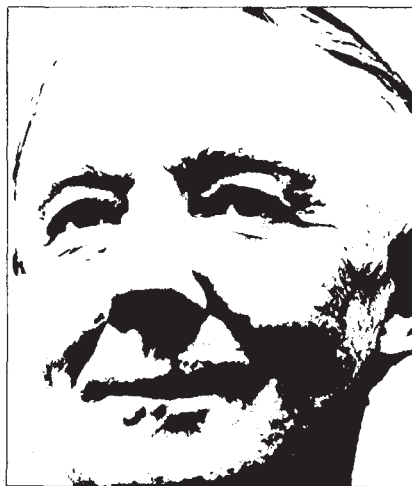
EVEN the sophisticated generally believe what they hear on the radio, or see on television. A few days ago these media prominently featured a report by the UK Department of the Environment which apparently dealt with the world's future. Listeners or viewers (I was both) received the impression that a group of economists and scientists had engaged in a complicated and detailed exercise, using sophisticated mathematical models and advanced computers, and after years of hard grind had come up with the conclusion that if the world's population continued to grow exponentially at something approaching 3%, in fifty or more years the forebodings of Malthus, first put forward nearly two hundred years ago, would be realised, and mass starvation would overtake mankind. Other shortages, including that of energy, would be less serious.

My reaction was that here was yet another expensive and pointless exercise, over-supported by government funds, discovering something which was obvious to anyone with a little knowledge of these problems. We have seen many such useless exercises in the past, the most notorious being a Cabinet Office report on Energy; this was obviously just another example of governmental irresponsibility wasting the taxpayers' money.

Before expressing this view, however, I waited until the next day, expecting to find a fuller summary in the serious daily press. I found no mention in the *London Times*, the *Financial Times*, the *Guardian* or the *Daily Telegraph*. I did not consult the tabloids. Now I've received the volume which stimulated the radio and television but did not interest the newspapers. It is Research Report 19 from the Systems Analysis Research Unit

(SARU) of the UK Departments of the Environment and Transport, and is called "Sarum 76: Global Modelling Project". It extends to some 230

Model report



KENNETH MELLANBY

pages, and as it costs no less than £15, is not expected to have a mass circulation.

My reactions to the document are quite different to those stimulated by the radio. It is no facile statement of the obvious. I assume that it did not interest the newspapers because it draws no conclusions, contains no pithy summary forecasting doom, but is a serious and careful study, adding another useful volume to the series of reports from these two government departments.

The writers of Sarum 76 (anonymously, only the Director's name appears) have got together an immense amount of information about

world incomes, populations and their changes, energy, renewable and non-renewable resources and agricultural productivity. The figures and tables alone make the volume worth its inflated price. The team has produced a sophisticated mathematical model which, if you think modelling is worthwhile, is itself a model (in another meaning of this over-used word) for this type of study, and it will clearly be useful for others with similar interests.

The report shows that Sarum 76 can be used to test many reasonable assumptions for the effects of any number of permutations and combinations of the changes in population, productivity, climatic conditions and all the other known factors which may affect our future. It makes no claims to foretell the future, and admits that this is not the ultimate, perfect model for this purpose. There is indeed a figure, reminiscent of some in *Limits to Growth*, which shows that if the world population grows at 2.27% a year, food supplies per head could increase for fifty years and then fall rapidly, but this should be seen in the context of many other possible forecasts.

We are now wedded to acronyms, but where those have existing meanings they should be used with care. ASH is undoubtedly suitable for Action on Smoking and Health, but in England Sarum is the old name for the city of Salisbury, and our Established Church adopted the Sarum Liturgy in 1542. Its use brought back to my mind a limerick—in England, incidentally, Hampshire is commonly abbreviated to Hants:

There was a young curate of Salisbury
Who used to be most harum-scarisburly
He went through Hampshire
Without any pampshire
'till his bishop said he must walisbury

correspondence

Research 'ridiculous'

SIR,—Regarding your editorial of 8 September, it seems to me that the Psychological Association and Dr Shockley and Dr Huxley have all missed the point. The proposed research may be desirable or deplorable, but above all it is ridiculous.

Dr Shockley proposes the existence of close linkage between the (unknown) polygenes for skin colour and the (unknown) genes that determine (unmeasurable) absolute intelligence. Even the existence of "absolute intelligence" is unproven and debatable.

For present day genetics and psychology to tackle this problem is like Antonie von Leeuwenhoek studying the structure of the ribosome. Maybe it will be possible some day, in the far future, by which time, hopefully, we will be getting excited about something other than race.

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Using hyphens

SIR,—I heartily endorse J. Faber's plea (19 May, page 202) that semantically essential hyphens be reinstated in compound adjectival phrases. The current practice of abandoning this punctuation nearly always generates serious ambiguity, or even utter gibberish. Thus, a printed label on a recent pharmaceutical product advises me: "Do not take with milk or calcium containing anti-acids." (In this instance one must presume the reference is to *low* calcium, since a recent report in a reputable journal refers to "high calcium containing phospholipids".)

Faber rather charitably attributes this widespread offence to "sheer sloppiness", but my experience has been that the problem stems largely from a deliberate editorial conspiracy. Critical hyphenation is commonly deleted between manuscript and galley, and frequently the deletion is enforced over the authors' protests concerning the resultant semantic atrocities. If one encounters the phrase "men chasing women" in print, the prevailing editorial policy makes it impossible to conclude which group of participants is pretending to be in flight.

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Solar nuclear waste disposal

SIR,—It has been recognised for some years that nuclear (fission) reactors are power sources for which there is an increasing need in view of growing world demands for power and depleted fossil fuel supplies. Such power reactors provide a reliable bridge to new power technologies, such as fusion and solar energy collection, which are under long term development.

However, serious questions are rightly asked about the real and imagined safety aspects of the increased proliferation and use of fission reactors of various types. One principal area of concern is the need to find acceptable and safe long term methods for disposal of spent nuclear fuel. Many have yet to be convinced of the conventional wisdom of burial of such wastes in deep ocean trenches or in deep stable rock formations.

In the light of our undoubted need for increasing access to nuclear power, coupled with parallel needs for safe disposal of nuclear wastes, I suggest that serious consideration be given to the application of existing space technology to transport such wastes to the sun.

Reliable space transport systems have been developed in a number of countries during the past two decades. They culminate in the Space Shuttle System. Within the next few years the Shuttle will be making regular, and almost routine, manned journeys between the earth and earth orbits to perform a wide variety of tasks. These include transport and operation of Spacelab, launch of satellites, recovery and manipulation of payloads in space using the Remote Manipulator Facility, deployment of Space Telescope and so on.

It is thus suggested that serious thought should be given, at an international level, to the possibility of using Shuttle or Shuttle technology to carry, on a regular basis, safely encapsulated containers of nuclear waste into temporary parking orbits above the earth from which they can be assembled into payloads for vehicles (also carried into orbit by Shuttle) to carry them on to the sun. The development of such a 'sun-bus' system including its propulsion and guidance components is well within the capability of contemporary space technology.

The writer does not overlook the

many economic and safety aspects of this proposal which will have to be considered in the development of this application of space technology to the service of all mankind. It would appear to be as attractive, if not more attractive, from many points of view, as the current terrestrial nuclear waste disposal methods which are used or which have been proposed. It has the strong advantage of not imposing a social burden lasting for many centuries for the monitoring and safeguarding of our legacy of nuclear waste.

R. W. NICHOLLS

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Farm energy

SIR,—In his recent contribution Kenneth Mellanby seems determined to show us that everything is for the best in the best (ours) of all possible worlds. When it comes to energy use in farming he should be more careful: one man's objective view is another's blind prejudice.

Although our use of energy in farming looks modest by comparison with our profligacy in other directions, it is monstrous by world standards. If all those bushmen, whose energy economy he derides, were to adopt our farming methods they would use all their countries' usual energy supply in the process. If they were foolish enough to adopt our food processing habits as well 40% of the global energy supply would be required.

Although it is possible for several people to be fed from 1 ha, farmed by modern methods, that is not what happens; something like 0.7 ha and half a tonne of oil a year are needed to feed each and every Briton. Of course we have got used to all this, and it would be politically awkward to change, but that is not to say we have a God-given right to carry on. As Gerald Leach says in his book *Energy and Food Production*, "this is quite clearly not a viable system for all time".

Finally, I cannot share Mellanby's optimism that we are responding adequately to the problem he seems so intent to minimise. The main driving force of technical change in food production is towards economy of labour use. I see no sign of significant reductions in energy requirements.

MICHAEL KNEE

Kent, UK

news and views

New look at the aminergic nervous system

from Key Dismukes

THE aminergic neurotransmitters—noradrenaline, dopamine, and 5-hydroxytryptamine—have been studied with an intensity far disproportionate to the tiny fraction of brain cells which contain them. This attention has been drawn by the realisation that many clinically important psychoactive drugs apparently operate by altering availability of one or more of these transmitters. New histochemical techniques for mapping aminergic projections are revealing striking anatomical features and hint at mechanisms by which these neuronal systems may shape emotion, vigilance, and learning processes. For instance, almost all of the brain's noradrenaline is contained in a few thousand neurones originating in tiny nuclei in the brain stem. These few cells, however, innervate the entire cerebral cortex and much of the underlying diencephalon, as well as the cerebellum. To accomplish this diffuse innervation, the axons from each noradrenergic neurone must branch perhaps a hundred thousand times.

Several laboratories have reported a startling feature of these ascending aminergic projections. Only about 5% of the axonal varicosities from which neurotransmitter would appear to be released are associated with postsynaptic neurones. The remainder lack the close cellular apposition, subsynaptic web and postsynaptic membrane thickening which characterise classical synapses in electron micrographs. The implication is that the majority of amine-containing varicosities release neurotransmitter which must diffuse to remote receptors, a mode of operation intermediate between the private addressing of classical synaptic messengers and the broadcasting of neuroendocrine secretion.

The technique used in these studies was adopted by the Descarries group at the Université de Montréal from a

procedure developed by Aghajanian at Yale University. Radiolabelled noradrenaline or 5-HT is topically applied (*in vivo*) to a region of the brain for just long enough to be preferentially accumulated by noradrenergic or serotonergic neurones. The tissue is then prepared for autoradiography and examined by electron microscopy. Specificity of labelling was verified by demonstrating that neuronal accumulation of label was blocked by inhibitors of specific uptake or by lesions which selectively destroy one class of neurones.

³H-noradrenaline applied to rat cerebral cortex was found to be taken up and distributed in noradrenergic neurones in a pattern closely similar to that of endogenous noradrenaline (Descarries *et al. Brain Res.* **133**, 197; 1977). The radioactive label dispersed along tiny diffusely projecting unmyelinated axons, which have small enlargements spaced at frequent intervals (1–3 μ m). Label was highly concentrated in these axonal varicosities, and was mainly associated with numerous small agranular synaptic vesicles. Although these varicosities have the appearance and inner structure typical of classical synaptic boutons, less than 5% showed signs of being connected to postsynaptic membranes (as opposed to 50% of unlabelled boutons in the surrounding neuropil). Labelled boutons making synaptic contact could not be distinguished morphologically from those without synaptic junctions.

Such non-synaptic boutons may turn out to be a common characteristic of brain aminergic systems. The axonal projections of cerebral serotonergic neurones closely resemble those of noradrenergic systems, with only about 5% of boutons marked by ³H-5-HT evincing synaptic contact (Descarries *et al. Brain Res.* **100**, 563; 1975). Chan-Palay has shown that the extensive plexuses of serotonergic axons lying on both sides of the ependymal walls of the ventricles, in the arachnoid sheath around cerebral blood vessels,

and in the pia over the spinal cord have numerous axonal boutons but lack post-synaptic structure (*Brain Res.* **102**, 103; 1976). Earlier approaches to tagging aminergic projections suggested the existence of non-synaptic varicosities in (presumably) dopaminergic endings in the striatum and in catecholamine and indolamine terminals of the median eminence (see references in Chan-Palay *op cit.*).

It is important to note that in none of these systems have the axonal varicosities yet been shown to release their sequestered amines. However, indirect evidence suggests that release does occur. The non-synaptic boutons have all the components normally associated with neurotransmitter secretion. Furthermore, it seems unlikely that the large fraction of aminergic neurotransmitter content which can be released by depolarisation *in vitro* or stimulation *in vivo* could be accounted for by the small fraction of boutons with synaptic contact. Release from non-synaptic boutons is known to occur in sympathetic neuromuscular junctions, such as in the vas deferens.

What is the functional significance of these non-synaptic axonal boutons? The lack of close apposition to postsynaptic membranes implies that receptors for released amines must be distributed farther afield than in classical junctions. 5-Hydroxytryptamine released into the cerebrospinal fluid of the ventricles could be carried to wide areas of the brain. Similarly, the diffuseness of ascending noradrenergic and serotonergic projections would allow distribution of aminergic neurotransmitters throughout the forebrain. Descarries and coworkers estimate that the cortex contains some 330,000 noradrenaline varicosities per mm³, with remarkably uniform distribution. Thus, every cell in the cerebral cortex may lie within less than 30 μ m of a non-synaptic noradrenergic bouton. This arrangement suggests that release of noradrenaline would alter the activity

of large populations of cells, rather than controlling individual targets on a one-to-one basis. Both Chan-Palay (*Cerebellar Dentate Nucleus* Springer-Verlag, 1977) and Descarries *et al.* (*op. cit.*) speculate that these aminergic systems may be used as neuromodulators, biasing the responses of target cells to classical neurotransmitters and shaping the activities of large neuronal regions.

Regardless of whether non-synaptic boutons turn out to release neurotransmitter, a neuromodulatory role for these diffuse aminergic projections is suggested, both by their anatomical arrangement and by neurophysiological data. Freedman and coworkers for example, recently reported that noradrenergic activation, although suppressing spontaneous firing of cerebellar Purkinje cells produced a relative enhancement of the Purkinje response to synaptic inputs from other cells (*Expl Neurol.* **55**, 269; 1977). Noradrenergic projections to the forebrain contrast with the fibre pathways of classic neuroanatomy not only in the diffuseness of their distribution, but also in topographic organisation. In classically described long pathways the terminal projections of individual cells

are generally arranged so that a map of the total projection field corresponds topographically with the distribution of cell bodies. R. Y. Moore and coworkers have found that this sort of organisation does not occur in noradrenergic projections from the locus coeruleus to the neocortex; rather, projections from a single cell are widespread, with vast overlapping (*A. Rev. Neurosci.* in the press). Interestingly, many of the brain regions receiving noradrenergic input make reciprocal connections back to the tiny locus coeruleus nucleus. These observations suggest that the diffuse ascending aminergic systems represent a distinct category of neuronal organisation.

A neuromodulatory role is concordant with a massive literature implicating amines in various aspects of behaviour. A leading biochemical theory of schizophrenia suggests malfunction of dopaminergic systems. Alterations in mood are correlated with neuronal availability of noradrenaline. Many studies have linked catecholamines and indolamines to vigilance, reward, and learning, yet it has not been possible to identify a specific aminergic role in any of these behavioural processes. That elucidation may now be a step closer.

Egg activation

from Duncan O'Dell

WHEN an egg is fertilised, many of its properties change promptly. Most species show an increase in the rate of respiration, protein synthesis is turned on, permeability and transport characteristics of the surface membrane change, a block to the entry of supernumary sperm is established and meiosis, where appropriate, is resumed. It has long been considered that during oogenesis an egg acquires all the machinery necessary to respond rapidly to fertilisation, but that these several responses become 'blocked' until they are 'activated' by the entry of a sperm. For many years, there has been a search for the 'switch' or 'switches' that would serve to activate eggs and explain why so many different processes get turned on more or less together. This search was complicated by the fact that some eggs, such as those of sea urchins, can be parthenogenetically activated by any of a long and confusing list of treatments whereas others, such as those of sea squirts, are refractory to most procedures tried. Matters became much clearer in 1974

when Steinhardt and Epel (*Proc. natn. Acad. Sci. U.S.A.* **71**, 1915) showed that the ionophore for divalent cations, A23187, would activate echinoderm eggs; subsequent work by these and other authors has shown both that many other eggs can be similarly activated and that the movement of calcium ions is intimately involved in the process.

In a more recent analysis (Johnson *et al.* *Nature* **262**, 661; 1976) the activation of sea urchin eggs was divided into two stages. The first, which occurs in the minute after fertilisation or activation, involves the exocytosis of the granules under the surface of the egg leading to various surface changes and it is this event that can be brought about by the calcium ionophore. The second stage, about three or four minutes later, involves the onset of macromolecular syntheses and the most interesting feature here is that the first stage is not a prerequisite for the second. A number of treatments—ammonia, procaine, nicotine—all lead directly to the metabolic activation of the egg. Johnson *et al.* showed that all these agents caused an efflux of H^+ ions with a subsequent rise in the intracellular pH of the eggs. If this efflux

of acid was blocked in various ways, the second stage of activation did not occur. It was then suggested that low intracellular pH served as the natural 'block' of metabolism before fertilisation, with the caveat that this sort of analysis has not yet been performed on many species of egg. Johnson *et al.* concluded by speculating that such shifts of intracellular pH could be regulating mechanisms elsewhere when quiescent cells resume division.

In this issue of *Nature* (page 590) Lopo and Vacquier take up the challenge presented by this speculation. First, the observation that intracellular pH rises after fertilisation is confirmed. But the changes in pH have also been followed for much longer during development, and it has been found that the higher pH of the newly fertilised egg is not maintained for long. Since the actively dividing cells of cleavage stage embryos seem to have an intracellular pH lower than that of the 'blocked' mature egg cell, it would seem that low pH alone is no absolute barrier to the processes involved in preparing for mitosis. The initial rise in pH, then somehow serves irreversibly to 'unblock' the egg. However there may still be a link between DNA synthesis and gross intracellular pH as Lopo and Vacquier show by use of procaine. This drug promotes DNA synthesis and a high intracellular pH; when procaine is removed, both fall.

It seems established, then, that at least in sea urchin eggs an early event following fertilisation is a rise in intracellular pH and that this rise is associated with the onset of the processes of early development, but the higher pH is not necessary for the completion of these processes. Clearly, there is scope for further analysis here. All these pH measurements were made on egg homogenates and subtle local variations within the cell would have been missed. The mode of action of a drug such as procaine is by no means exactly certain. More work needs to be done before one can interpret the consequences of a rise in intracellular pH with confidence. However, the past three years, starting with Steinhardt and Epel's experiments on A23187, have taught us a great deal about fertilisation and egg activation. Although the definitive analysis is not yet quite in sight important experiments are being done and there is a feeling that an interpretation will be made. At the beginning of this century good scientists worked hard on the problem but mainly produced a baffling list of egg activating treatments, there is now hope that the scientific great-grandchildren of pioneers like J. Loeb will be able to interpret that list in terms of calcium levels, pH shifts, surface membrane changes and so on. □

Ciliar dyneins

from Roy Burns

THE similarities between the molecular basis of muscle contraction and the beating of cilia flagella have been evident for several years. Both mechanisms involve the sliding of parallel filaments, resulting from the hydrolysis of ATP by enzymes which transiently form crossbridges between the adjacent filaments: the myosin heads in the case of muscle and the dynein-containing side arms of cilia and flagella. Furthermore, the demonstration by Gibbons and Gibbons (*J. Cell Biol.* **63**, 970; 1974) that the crossbridges of sea urchin sperm flagella are frozen when the ATP concentration is suddenly lowered, resulting in rigor, strongly suggests that the dynein-ATPase crossbridge cycle resembles the Lymn-Taylor cycle of muscle (see *News and Views* **235**, 362; 1972). It is however dangerous to extend the analogy too far, and Warner, Mitchell and Perkins (*J. molec. Biol.* **114**, 367; 1977) have recently shown that the outer sidearms of cilia are structurally much more complex than the myosin head crossbridge.

Each of the outer doublets of cilia and flagella bear a pair of sidearms which tangentially approach the neighbouring doublet. These sidearms can in general be removed by dialysis against low ionic strength buffer, and the outer sidearm selectively extracted with 0.5 M KCl (Kincaid, Gibbons & Gibbons *J. supramolec. Struct.* **1**, 461; 1973), and the principal component present in such high salt extracts from sea urchin sperm tail axonemes is the high molecular weight ATPase termed dynein-A. Warner *et al.* have examined such a preparation of dynein-A from cilia from both *Tetrahymena* and the gills of the lamellibranch *Unio* by SDS-gel electrophoresis and by negative staining.

SDS-gel electrophoresis of the *Unio* gill axonemes shows two principal high molecular weight bands, present in a 2:1 ratio, and four additional components with molecular weights greater than 300,000 daltons. Extraction with high salt selectively removes approximately half of the major component (Band-3) which has an estimated molecular weight of 360,000 daltons. When the high salt supernatant from either *Unio* or *Tetrahymena* is examined by negative staining, three principal structures are observed: a 9.3 nm spherical particle with or without a centrally located accumulation

of stain, and aggregates of 2-6 of these 9.3-nm subunits. A fourth unrelated particle is occasionally observed in the *Unio* preparations with dimensions of 9.4×12.7 nm together with some solubilised tubulin and some unidentified 200×3.5 nm filaments. The 9.3-nm particle is consistent with a protein of molecular weight 348,000 daltons, and in view of the extraction conditions is considered to be the monomeric form of dynein-A.

This particle is, however, significantly smaller than the attached sidearm, which in *Tetrahymena* is hook-shaped and measures $20-22 \times 8-10$ nm (Allen *J. cell Biol.* **37**, 825; 1968). Warner *et al.* find that the outer sidearm can be resolved by negative staining into at least three morphological subunits, with a centre-to-centre spacing of 7.5 nm. What then is the relationship between these smaller subunits and the 9.3-nm extracted particles? Three possibilities are suggested; first, that the outer sidearm is composed of one or more non-enzymatic molecules with an identical molecular weight to dynein-A. This possibility should not be discounted as the resolution of SDS-gel electrophoresis is low in the high molecular weight range ($\pm 10,000$ daltons), and the high salt extraction removes only half of the Band-3 component, the remainder presumably being a different protein with the same molecular weight. The second possibility is that the sidearm is a polymer of the 9.3-nm particle. In order to account for the lower centre-to-centre spacing of the subunits of the intact arm it is necessary for a conformational change to occur during extraction, or for the subunits to be superimposed *in situ*, and indeed the sidearms are tilted towards the base at an angle of 28° from the perpendicular. The third possibility is that only the distal portion of the sidearm is extracted, the proximal part remaining attached to the outer doublets but in a form undetected by either negative staining or conventional electron microscopy. However, all of these alternatives indicate that the sidearm is much more complex than the myosin head, and support the view that although the two contractile mechanisms share certain properties, they are unrelated.

Finally, Warner *et al.* have numbered the *Unio* and *Tetrahymena* high molecular weight axonemal proteins 1-6, while Gibbons *et al.* (in *Cell Motility, Cold Spring Harbor Conferences on Cell Proliferation* (eds Goldman, Pollard & Rosenbaum) **3**, 915; 1976) had labelled the sea urchin proteins Band-C, A, D, and B, where Band-A corresponds to dynein-A (also termed dynein-I) and to Warner's

Band-3. At first sight this suggests a proliferation of cumbersome nomenclature. Two points should be made: first, Gibbons has defined the dyneins as high molecular weight (above 300,000 daltons) microtubule-associated ATPases so that certain of the sea urchin high molecular weight proteins, for example Band-B, which lack ATPase activity are not by definition dyneins. Second, it is clear that the same experimental procedure does not necessarily extract the same components from all cilia and flagella; for instance the cilia and flagella of *Aquipten* differ in their extraction properties (Linck *J. Cell Sci.* **12**, 951; 1973) and not all species of sea urchin behave identically. It is probably wise therefore to retain separate nomenclatures until the function and location of each of the high molecular weight components is clear. The differences in the extraction of different cilia and flagella suggest also that the elucidation of the structure and mechanism of movement may be hastened by exploiting obscure organisms rather than confining our attention to a single widely available species. □

Coulson rules OK

from John Walker

THE problem of calculating the electronic energy levels of defects in semiconductors has not been solved, and is currently the subject of much controversy; some new results and reinterpretations of data on point defects in diamond by Gordon Davies (*Nature* **269**, 498; 1977) strongly suggest that the method of calculation proposed by Coulson as far back as 1957 (Coulson & Kearsley *Proc. R. Soc. A* **241**, 433; 1957; see also Coulson & Larkins *J. Phys. Chem. Solids* **32**, 2245; 1971) is the most appropriate one.

Since the 1920s and the development of quantum mechanics it has been possible to calculate with reasonable (and in some cases extreme) accuracy the electronic energy levels of atoms and molecules, and hence their characteristic spectra. The calculations are simplified because an atom is an isolated system; the electrons do not interact with other atoms. Molecules are more difficult, but only a few atoms are involved, at least in the case of simple molecules.

In a solid the analogous problem to the hydrogen atom is an electron bound to a lattice defect such as a vacancy. Here the situation is complex; the surrounding lattice atoms must be taken into account, since they are

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intimately connected to the defect by chemical bonds. They affect the magnitude and symmetry of the potential seen by the electron, and this influence is complicated by the fact that they can vibrate and become distorted.

In semiconductors there are simplifications in some cases—the so-called 'shallow' centres—whose energy levels lie close to the edges of the forbidden gap. These can be treated in a hydrogenic approximation. For 'deeper' levels the approximate treatment does not work, and diamond is particularly difficult because its forbidden gap is wide (which is why diamond is transparent) and all levels are deep.

On the other hand, a diamond is effectively a giant in theoretical chemistry? So why not apply to it the techniques used in theoretical chemistry? This is what Coulson and Kearsley did. They treated the neutral vacancy (a lattice site from which the carbon atom has been removed) as a 'defect molecule' containing four electrons—the 'dangling bonds' from the four nearest-neighbour carbon atoms which were broken in creating the vacancy. (For a positive or a negative vacancy there are three or five electrons.)

The calculations predicted a 1E groundstate with a 1T_2 state about 2 eV higher in energy. (E and T_2 denote doublet and triplet orbital states. The superscript $=2S+1$, where S is spin, as in atomic spectroscopy. Hence S is zero in this case.) The transition 1E to 1T_2 is allowed. It so happens that an absorption band in irradiated diamond—the GR1 band—occurs at about 2 eV and has a non-paramagnetic ($S=0$) ground state. Since irradiation produces vacancies it was reasonable to assume that this absorption band was due to the neutral vacancy. Experimental work 16 years later (Clark & Walker *Proc. R. Soc. A* **334**, 249; 1973; Davies & Penchina *Proc. R. Soc. A* **338**, 359; 1974) confirmed that the GR1 transition was indeed between an E ground state and a T excited state. So the Coulson calculations seem to work.

In his paper Davies has suggested that the calculations work equally well for the negatively-charged vacancy (V^-). He has pointed out that irradiation of some diamonds (the so-called type Ib) gives a much weaker GR1 band than normal, but produces very strong absorption in the ultraviolet—the ND1 band. Since it is unreasonable to assume that irradiation produces vacancies in some diamonds but not in others, Davies argues that they are being produced in a different charge state. Type Ib diamonds contain nitrogen, which should be a donor, so vacancies are likely to be in the

negative rather than the neutral charge state. The calculations predict that V^- probably has a 4A_2 ground state, with an allowed transition to a 4T_1 excited state several eV higher in energy. Earlier work (Davies & Lightowlers *J. Phys. C: Solid State Physics* **3**, 638; 1970) has shown that ND1 is indeed due to an A to T transition and that the defect is tetrahedral, as one would expect for a vacancy. Furthermore, Dyer and Du Preez (*J. chem. Phys.* **42**, 1898; 1965) have demonstrated that ND1 is paramagnetic, which it would be with a 4A_2 ground state. They have also shown that the ND1 absorption can be strengthened, and GR1 reduced, or *vice versa*, by shining light of appropriate wavelengths on the

diamond. This phenomenon is easier to explain if ND1 and GR1 are different charge states of the same defect, rather than being different defects, as was formerly assumed.

Davies's suggestions therefore fit the facts. They indicate that further work is desirable on the ND1 centre in EPR. They imply that the sign of charge carriers involved in photoconductivity at ND1 and GR1 should differ, which can be checked. And most important of all, they imply that the 'defect molecule' approach of Coulson and his coworkers is the best way to calculate the electronic properties of defects in semiconductors. It is to be hoped that Davies's work will stimulate further activity amongst the theoreticians. □

Primaeval heavy leptons

from P. C. W. Davies

DISCOVERIES in subatomic particle physics take place against a continual struggle for more technology and money. The Universe enjoys complete freedom from these worries and produces for free events which may tax human resources to the limit. During the big bang, exceedingly high temperatures and energies stimulated subatomic processes of all varieties, some of which have left relics for us to observe today, so it has become increasingly attractive to confront the theoretical work on these processes with astrophysical and cosmological, as well as technological, data.

A famous example of this approach concerns the unknown rest mass of the neutrino, which most physicists would like to be zero. Laboratory experiments are not particularly sensitive to the neutrino mass, but cosmology is. A study of the fireball resulting from the big bang indicates that the entire Universe should be filled with primaeval neutrinos, about three hundred of them per cm^3 —a billion times more numerous than atoms. The existence of a similar background of photons was confirmed by observation in 1965, but neutrinos interact so weakly with matter (it would take a lump of lead several thousand light years thick to absorb them) that their presence has remained undetected. Nevertheless, their energy will gravitate like any other, and thereby influence the characteristics of the expanding Universe. If they do possess even a tiny rest mass, they are present in such profusion that the cumulative gravity of all the

neutrinos could dominate the cosmological motion. It has been estimated that a neutrino mass in excess of 40 eV (about 10^{-4} of the electron mass) would be sufficient to cause the expansion of the Universe to decelerate more rapidly than observations permit, and eventually to bring about its demise by gravitational collapse.

In two recent papers in *Physical Review Letters*, similar arguments have been used to place mass limits on a hypothetical new particle: a neutral heavy lepton. This entity is predicted by some recent gauge theories of weak interactions and is being actively sought by particle physicists. Its properties would be rather like those of a super-heavy neutrino. If these particles exist, they would have been present along with their antiparticles in the primaeval fireball, but their high mass would have greatly reduced their abundance over that of ordinary neutrinos. In the first paper, the late Benjamin Lee of Fermilab, and Steven Weinberg of Harvard University (currently on leave at Stanford) consider the thermal equilibrium abundance of the heavy leptons when the temperature of the Universe was about 10^{10} K (about one second after the big bang). Before this point the cosmic matter density was so high that even the feebly-interacting neutrinos would have felt its presence and been held in thermal equilibrium with it. This fact enables the abundance of neutrinos and other particles to be calculated from the common temperature of all the primordial material. However, when it comes to calculating the abundance of the heavy leptons, it turns out that their density would have been so low that their natural incli-

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A hundred years ago

THE GELADA.—Several living specimens of this extremely rare Abyssinian monkey first described by Dr Ruppell in 1835, have quite recently reached this country for the first time and are being exhibited at the Alexandra Park. The exact affinities of the species have never been fully determined, different biologists placing it, some with the Macaques, others with the Baboons. It is peculiar in that the male is covered with very lengthy hair like that of the Wanderoo, whilst the female is a much more ordinary-looking monkey. In the male, also, there is a bare spot, in shape like an inverted T, upon the breast which is of a light-pink colour, becoming red and expanded into an inverted heart-shaped patch upon excitement. The tail is long and like that of a lion, having a bushy tuft at the extremity. The colour is a sooty dark-grey brown, verging upon black; the hands and face are black; the nails are powerful and long. The size of the male is about that of a Chimpanzee four years old. The eyes are close together, and the snout prolonged. The living animal has a habit of everting the whole upper lip when irritated and thus exposing its formidable array of teeth.

From *Nature* 16, 11 October, 512; 1876.

nation to annihilate with their antiparticles was inhibited, simply due to the rarity of particle-antiparticle encounters. Consequently, relatively more of them would have escaped this fate and survived to populate the Universe. Using a simple model of the primaeval fireball, Lee and Weinberg are able to show that the mass of these heavies must be at least about 2 GeV (four thousand times the mass of the electron) to avoid the gravity of this residue drastically decelerating the Universe. Curiously, the same argument which places an upper bound on the neutrino mass places a lower bound on the heavy lepton mass. The reason for this is that the heavier the particles the more they gravitate individually, but the less abundantly they are produced at a given fireball temperature. Because of these competing effects, the combined gravity of all the particles is greater if either the mass is especially low or high—very many light particles or a few very heavy ones. In the neutrino case, we know from laboratory experiments that the mass is tiny, so the individual-gravity factor dominates,

but the reverse is true of the heavy lepton, and it is the abundance factor which is more important.

A fascinating consequence of this calculation is that the abundance of these particles, if they exist and are not too massive, could still be high enough to contain nearly all the mass of the Universe, which opens up the prospect of dozens of times more matter in the form of weakly interacting, virtually unobservable, heavy neutrinos than in all the galaxies. If this is correct, then it might indeed be sufficient to cause the cosmological expansion eventually to reverse into collapse.

In the second paper, Duane Dicus and Edward Kolb of the University of Texas at Austin, and Vigdor Teplitz of the Virginia Polytechnic Institute consider how the Lee-Weinberg result would be modified if the heavy neutrinos are weakly unstable, and decay after a while into ordinary neutrinos. The essence of their calculation is the observation that cosmic neutrinos resulting from recent heavy lepton decays are more energetic, and hence gravitate more strongly, than the primordial ones, because the latter have been redshifted to low energies by the expansion ever since the big bang. Hence the cosmological measurements provide a limit on the lifetime of the lepton. However, this quantity

can also be inferred from the theory of weak interactions if the mass of the lepton is known. Turning the argument round, the lifetime limit can be used to place an upper bound on the mass, which works out at about 10 MeV, or less than one hundredth of the Lee-Weinberg value for a stable heavy lepton. The particle lifetime corresponding to this is about a year.

The authors have checked that any high energy photons also produced in the decay events during the first few hundred million years would have been degraded (thermalised) by scattering. Those produced after this would be swamped by the cosmic X-ray background, so are not detectable. Nor do the energetic decay neutrinos affect the nucleosynthesis in the primaeval fireball, because at 200 s this process occurs too early for the decay products to have accumulated significantly. Unfortunately, the presence of the decay neutrinos, in spite of their higher energy, is still too ephemeral to register in neutrino detectors or noticeably to affect protons in cosmic rays so that, intriguing though these ideas are, no positive experimental verification of cosmic heavy leptons appears likely. Nevertheless, the imposition of mass bounds at least lets the laboratory physicist know what to expect from terrestrial ones. □

What future for Antarctic geology?

from Peter Barker

The 3rd Symposium on Antarctic Geology and Geophysics held at Madison, Wisconsin, on 22–27 August, was cosponsored by SCAR, IUGS and ICG. The organising committees, chaired by Campbell Craddock (University of Wisconsin-Madison), ensured a friendly and interesting meeting.

ANTARCTIC Earth science can be a slow, difficult business, at sea as well as on land. The weather is often foul and the ratio of outcrop to ice vanishingly small. The great expense of putting a party into the field can generate years of delay, and tends particularly to discourage the return visit which a mature assessment of the first season's work often recommends. Along with delay, these conditions breed unconscious error, over-reached speculation and a parochial viewpoint, and ensure their prolonged survival in the literature. Nevertheless, the past few years have seen a revolution in the earth sciences generally, and considerable

funds allocated for Antarctic research. The importance of the symposium at Madison, therefore, lay not in its being larger, better attended and broader in scope than its predecessors at Oslo (1970) and Cape Town (1963), but in the opportunity it provided to assess the quality and global relevance of contemporary Antarctic earth science, and consider its future.

Continental geology, the traditional core topic, occupied less of the programme and produced few of the kind of surprises which must have animated past meetings: the heroic age of exploration and reconnaissance mapping is past, the emphasis shifted towards remapping anomalous areas and questioning the reality of the classically-defined widespread orogenic episodes (in North Victoria Land, for example). Better descriptions of the oldest Antarctic rocks and more confident (although conflicting) comparisons with neighbouring Gondwana continents

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were evident than at Oslo, but for most of Antarctica the isolated coastal exposures and ice-covered interior seem a crucial impediment to further understanding. Radar ice-thickness measurements are revealing something of sub-ice morphology and, in those rare cases where allied geophysical data are available, seem capable of generating some small, speculative understanding of the interior. There is no doubt, however, that the geology of most of Antarctica, East and West, and its important glacial history, will remain obscure without an expensive and time-consuming programme of survey-controlled, regional sub-ice drilling.

Perhaps the liveliest part of the symposium, in contrast, was a two-day section on the geodynamics of the Scotia Arc. Considerable progress has been made since 1970, particularly in understanding southern South America's evolution and strengthening its connection with South Georgia. A Cretaceous age for almost all the Scotia Sea is now established, but the earlier history of the region remains uncertain. A crucial unknown, the positions of the Antarctic Peninsula and South America at the Gondwanaland margin, should emerge shortly; magnetic anomaly-based Gondwanaland reconstructions at Madison were conflicting, but obviously convergent, and the growth of marine geophysical interest in the Weddell Sea should complete the puzzle.

Under the influence of geodynamics, some Scotia Arc studies are oriented towards the processes involved in formation. The area is particularly well-endowed with back-arc basins, and a geochemical data base for these and other active margin igneous processes as broad as any in the world is now available.

Mineral resources, presently occupying the attention of Antarctic Treaty signatories were considered in only six out of about 150 papers. This dearth does not reflect wilful ignorance (the academic ostrich!), nor in the informal opinion of the meeting does it hide a much greater actual knowledge. Simply, reconnaissance mapping is complete and any resource detectable by such means is known; none is economic to extract at present, and such more detailed, follow-up surveys as have been carried out have proved no more fruitful. Offshore petroleum prospects were not discussed at Madison but this case is essentially similar, although there has been less reconnaissance.

The main goal of Southern Ocean marine geology (a topic hardly considered at Oslo) is an understanding of the interdependence of climate, circulation, sedimentation and Antarctic

Have gunk, will travel

from Peter J. Smith

A *nuée ardente* is a rapidly flowing, turbulent and often incandescent cloud of gas and ash originating in an explosive volcanic eruption. The first one to come to the attention of scientists, but surely not the first ever to be seen, was that from Mt Pelée (Martinique), which in 1902 overwhelmed the town of St Pierre, killing all but two of the inhabitants. This still remains the most famous of all, although many others, including much larger ones, have been observed since, not least from Mt Pelée itself.

But observation is one thing and scientific investigation is another; by their very nature *nuées ardentes* in action (as opposed to the deposits they leave behind) are not particularly amenable to the latter. The pioneer in this field was Frank Perret, who studied hundreds of *nuées ardentes* at close quarters after the eruption of Mt Pelée in 1929, captured their visual beauty in a remarkable series of photographs, and almost ended as a victim of one. Today, however, some aspects of glowing clouds may be studied from the relative safety of an aeroplane, which is how Moore and Melson (*Bull. Volcan.* 33, 100; 1969) observed the 1968 eruption of Mt Mayon (Philippines) and how Stith *et al.* (*Geophys. Res. Lett.* 4, 259; 1977) approached the eruption of St Augustine Volcano (Alaska) in 1976.

One of the most conspicuous characteristics of a *nuée ardente* is, of course, its speed, which reference books usually give as "perhaps 100 kilometres an hour" or something equally vague but which Stith and his colleagues have now determined much more accurately using aerial photography. Thus the *nuée ardente* produced by St Augustine Volcano on 8 February 1976 began moving down the initial 1:3 slope with a speed of 180 km per hour, slowed to 75.6 km per hour down the subsequent 1:7 gradient, then to 46.8 km per hour (1:12) and finally to 21.6 km per hour (1:28) before entering the sea 6 km from the volcanic cone. The average speed was 54.0 km per hour, which means that the 6 km was covered in less than 6.7 minutes.

The chief reason for such astonishing speeds is the 'fluidisation' of the volcanic particles brought about by the release of gases, a process which greatly reduces the frictional resistance of the mass as it moves under gravity. The dangers are obvious. A *nuée ardente* travels not only rapidly but with very little noise. A community in its path may be overcome without ever knowing what happened. □

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glaciation in a ocean basin of changing shape and with the most vigorous oceanic current system in the world. At Madison, papers based mainly on Deep Sea Drilling data and the Eltanin piston core collection attempted partial analyses of some of these relationships over time intervals varying from 1,000 years to 150 Ma. Individual papers were interesting but the pervading impression was of a huge field largely unexplored, with the gaps between contributions at least as important.

The four disparate fields considered above, with very few exceptions, contain all that is of wider interest in Antarctic earth science at present. They are at very different stages of development, from youth to old age, and their futures too are likely to diverge. Marine geology should prosper independently, with the driving force of the Southern Ocean's importance to world climate and primary productivity, and the basic unity of circum-Antarctic circulation as a constraint on the multi-disciplinary approach. One looks, perhaps, for an

injection of numerical modelling, to link through the huge range of periodicities being examined and to direct further sampling.

The future of Antarctic geology is less certain. Some aspects, such as Scotia Arc studies, may continue to develop and, as the emphasis in geology changes, remain as fertile ground for the study of processes. For much of Antarctica, however, a similar transition from unmapped wilderness to geological laboratory may not be possible; without sub-ice drilling too little of the context may ever be learnt. The value of Antarctica to global geology will then decline. Economic geology has reached a similar impasse. Workable deposits within and around Antarctica must exist and, given the increasing world pressure on known resources, continued interest is inevitable. There are no easy, short-term prospects, however, so the opportunity is still there for a balanced, disinterested political decision on the course of further exploration and exploitation. □

articles

Upper limits on the Faraday rotation in variable radio sources

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Data on the linear polarisation of variable radio sources are examined for evidence of a variable component of Faraday rotation. Strong upper limits are set, and these are shown to have profound implications for the physics of these sources. It is suggested that the electron energy spectrum is a relativistic Maxwellian rather than a power law. This may be consistent with the relativistic blast wave models of Blanford and McKee.

COMPACT radio sources which contain appreciable quantities of non-relativistic electrons will exhibit Faraday rotation and Faraday depolarisation at long wavelengths. The rotation at a frequency ν is

$$\chi = \frac{e^3}{2\pi m^2 c^2} \frac{n_c H \cos\theta}{\nu^2} l \text{ rad} \quad (1)$$

where n_c is the density of cold electrons, H is the magnetic field which makes an angle θ with the line of sight, and l is the thickness of the source along the line of sight. When $\chi \sim$ a few radians, the degree of linear polarisation drops to a small value¹.

If a radio source expands conserving magnetic flux, then $\chi \propto$ radius⁻⁴. Thus a variable radio source may exhibit variable Faraday rotation. If observations are made at two or more frequencies then changes in Faraday rotation can in principle be separated from changes in the intrinsic angle of polarisation. In this way, upper limits on the observed Faraday rotation can be used to place stringent upper limits on the density of cold electrons in compact radio sources.

The results of four years of monitoring the flux density and linear polarisation of 82 compact extragalactic radio sources were reported by Altschuler and Wardle², and an analysis of the data was given by Altschuler and Wardle³. Thirty-two of the sources showed distinct variability in their polarisation. Since the observations were made at two frequencies (8,085 MHz and 2,695 MHz), the data can be examined for the presence of Faraday rotation.

An appreciable variable component of polarisation was observed in many sources at 2,695 MHz, suggesting that Faraday rotation is often not large at this frequency. We have used this to derive a crude upper limit on the cold electron density³, and we found that the ratio of the densities of relativistic to cold electrons was typically in the range $n_{rel}/n_c \sim 10^4 \pm 4$.

Here the data are examined more carefully and the limits on the density of cold electrons are improved by an order of magnitude. This is shown to have profound implications for the physics of compact radio sources. The data suggest that the energy spectrum of the radiating electrons may be a relativistic Maxwellian rather than a power law. Almost identical conclusions have been reached independently by Jones and O'Dell⁴.

The observational data

In practice it is not a trivial task to isolate effects due to Faraday rotation from the data. There are several reasons for this: the variations in polarisation are rapid and intricate and are often poorly correlated between the two frequencies; there are few well defined simple outbursts; and, perhaps most important, the variable component is superimposed (vectorially) on a constant or slowly varying component of comparable amplitude (see refs 2 and 3).

We cannot determine the polarisation of the underlying component with much confidence in any case, so a simple statistical approach will be used. Of the 32 sources (from ref. 2) that exhibit variable polarisation, 16 are clearly variable in the degree of polarisation at both frequencies. In four cases the variations are distinctly uncorrelated between the two frequencies, and these sources have been dropped from the sample. For the remaining 12 sources the fluctuations in the degree of polarisation are fairly large and reasonably similar at both 8,085 MHz and 2,695 MHz, suggesting that the sources are optically thin much of the time, and that we are looking at the same piece of the source at both frequencies. The 12 sources have been selected without regard to the behaviour of the position angle of polarisation or the total flux density.

0851+20 (OJ287) is particularly important. In March 1974, the polarisation jumped at both frequencies from less than 2.5% to over 12%. Here the source is undoubtedly optically thin. Also, the variable component is so strongly polarised that the effect of any underlying constant component is very small.

If there is an appreciable variable component of Faraday rotation in these sources, we would expect to see changes in the position angle of polarisation at 8,085 MHz that are echoed at 2,695 MHz with an amplitude nine times larger. Inspection of the data in ref. 2 reveals no obvious cases of such behaviour. Usually the observed changes in position angle are either quite well correlated between the two frequencies, or else they are larger at the higher frequency.

Rough upper limits on the amount of Faraday rotation can be placed in the following way. For each day's observations, we take the difference between the position angles at the two frequencies, that is $\Delta\chi = \chi_{2,695} - \chi_{8,085}$. We then compute the mean and standard deviation of $\Delta\chi$ for all the observations of each source. (For 0851+20, we have used only the data between March 1974 and June 1975). The mean, $\langle\Delta\chi\rangle$, represents a constant component of Faraday rotation. This may be attributed to Faraday rotation both in our own Galaxy, and in whatever structure surrounds the variable radio source. The standard deviation, $\sigma\Delta\chi \times 9/8$, is a rough upper limit on the r.m.s. Faraday rotation at 2,695 MHz inside the variable component.

Two corrections should be made to this estimate. First, the average errors in the position angle measurements, $\langle\epsilon_{8,085}\rangle$

and $\langle \varepsilon_{2,595} \rangle$, should be subtracted quadratically. Second, the effect of superimposing the polarisation of a constant component is to reduce the observed changes in position angle. This can be compensated for, to first order, by multiplying $\sigma\Delta\chi$ by a factor $(\bar{m}+m_v)/m_v$, where $\bar{m}$ is the average degree of polarisation of the source, and m_v is the r.m.s. amplitude of the variable component, both at 2,695 MHz. The upper limit on the r.m.s. variable Faraday rotation at 2,695 MHz is then:

$$\sigma'\Delta\chi = \frac{9}{8} \left(\frac{\bar{m}+m_v}{m_v} \right)_{2,695 \text{ MHz}} (\sigma\Delta\chi^2 - \langle \varepsilon_{8,085} \rangle^2 - \langle \varepsilon_{2,695} \rangle^2)^{\frac{1}{2}} \quad (2)$$

The results are presented in Table 1 for the 12 sources in our sample.

It can be seen that in most cases $\sigma'\Delta\chi$ is of the order of half a radian, and in two cases is considerably less. These are, however, probably gross overestimates of the typical variable component of Faraday rotation in these sources. First, in each case (except 0851+20) we have used all the data, averaging over several outbursts. Presumably at certain times the sources may be opaque, leading to fluctuations in position angle that are not correlated at the two frequencies. Second, where there is limited data, or the variable component of polarisation has a large amplitude, then the amplitude of the underlying component may be badly overestimated, and hence too large a correction is applied to the observed values of $\sigma\Delta\chi$ in column (3). Third, in five out of the seven cases where $\sigma\Delta\chi$ is greater than 10° , inspection of the data shows that it is the position angle at the higher frequency that is showing rapid fluctuations, and is, therefore, unlikely to be due to Faraday rotation. In support of the first two points, for 0851+20 where it is certain that the source is optically thin, and where the effect of an underlying constant component is very small, we find $\sigma'\Delta\chi \lesssim 1/8$ radian.

We have found no evidence for variable Faraday rotation in these sources. From the above discussion, the typical amount of variable rotation at 2,695 MHz is most unlikely to be more than a few tenths of a radian. In the calculations that follow, however, we shall use the conservative upper limit of 0.5 radians.

Calculations

The Faraday rotation by cold electrons of density n_c is given by equation (1). The density of relativistic electrons can be estimated from the optically thin flux density. The emission coefficient for synchrotron radiation is given by⁵

$$\varepsilon_\nu = c_5(p)N_0(H \sin\theta)^{(p+1)/2} \left(\frac{\nu}{2c_1} \right)^{(1-p)/2} \quad (3)$$

where the electron energy spectrum is of the form

$$N(E) dE = N_0 E^{-p} dE, \quad E_i < E < E_j \quad (4)$$

The optically thin brightness temperature is

$$T_b = \frac{c^2}{2k\nu^2} \varepsilon_\nu \quad (5)$$

The number density of relativistic electrons is

$$n_{rel} = \int_{E_i}^{E_j} N(E) dE = \frac{N_0}{p-1} (\gamma_i mc^2)^{1-p} \left[1 - \left(\frac{\gamma_j}{\gamma_i} \right)^{1-p} \right] \quad (6)$$

where $\gamma = E/mc^2$.

Combining these we obtain

Table 1 The observed limits on Faraday rotation, at 2,695 HMz in 12 variable radio sources

Source	$(\Delta\chi)$ ($^\circ$)	$\sigma\Delta\chi$ ($^\circ$)	$\sigma'\Delta\chi$ ($^\circ$)
0300+47	15	3	<15
0605-08	64	13	39
0735+17	5	7	<22
0851+20	23	6	8
1055+01	-42	7	35
1334-12	-50	19	45
1510-08	-7	16	39
1641+39	26	12	81
1749+09	35	34	49
1921-29	-5	7	25
2155-15	21	13	32
2200+42	67	27	33

$$\frac{n_{rel}}{n_c} = \frac{4c_1}{c_5} mce^2 \frac{3^{-(p+1)/2}}{p-1} \frac{kT_b}{\chi \tan\theta} \left(\frac{\nu}{\nu_{B1}} \right)^{(p-1)/2} \times \gamma_i^{1-p} \left[1 - \left(\frac{\gamma_j}{\gamma_i} \right)^{1-p} \right] \quad (7)$$

$$\text{where } \nu_{B1} = \frac{eH}{2\pi mc} \sin\theta.$$

A lower limit on n_{rel}/n_c is obtained by setting $\nu = \gamma_i^2 \nu_{B1}$. If $p > 1$, we allow $\gamma_j \rightarrow \infty$ for simplicity then

$$\frac{n_{rel}}{n_c} \geq \frac{T_b}{\chi \tan\theta} \times \begin{cases} 1.5 \times 10^{-10} \ln \gamma_j/\gamma_i, & \text{if } p = 1.0 \\ 4.9 \times 10^{-10} & p = 1.5 \\ 3.1 \times 10^{-10} & p = 2.0 \end{cases} \quad (8)$$

T_b is typically observed⁶ to be in the range 10^{11} – 10^{12} K. In the previous section we derived upper limits on χ of a few tenths of a radian at 2,695 MHz. Hence for all sources that show variable polarisation at 2,695 MHz, we find $n_{rel} \gg n_c$.

This limit is independent of the magnetic field strength and the source dimensions. This is important since it makes the argument independent of the distance to the source, and also independent of the path length through the source, which may not be observable for example in the case of a thin shell geometry.

Lower limit on the relativistic electron energy spectrum

The limits we have derived on the density of cold electrons are so severe that we must also consider Faraday rotation by the relativistic electrons themselves. This is dominated by the lowest energy electrons and therefore can lead to limits on the presence of low energy relativistic electrons. Faraday rotation by an electron with a Lorentz factor γ is roughly proportional to γ^{-2} . (This is easily understood since in the rest frame of the electron, the radio photon is increased in frequency by a factor $\sim \gamma$.) For a power law electron energy spectrum, the equivalent density of cold electrons is^{4,7}

$$n_c(\text{equ}) = \frac{(p-1)(p+2)}{(p+1)} n_{rel} \frac{\ln \gamma_i}{\gamma_i^2} \quad (9)$$

where γ_i is the low energy cut off in the electron energy spectrum. Combining (9) with equations (1), (3) and (5) we find

$$\chi = A(p) \frac{\ln \gamma_i}{\gamma_i^2} \left(\frac{\gamma}{\gamma_i} \right)^{p-1} kT_b \cot\theta \quad (10)$$

where
$$A(p) = \frac{(p+2)3^{(1-p)/2}e^3}{(p+1)c_6(p)\pi m^2 c^4}$$

If the source becomes optically thick at a frequency close to 2,695 MHz, then $\gamma \sim \gamma_n \sim 10^{-9} T_b$ (ref. 8). Setting $\chi < \frac{1}{2}$ and $\theta = 45^\circ$, we obtain the limits on γ_i listed in Table 2.

Since these are strong lower limits on γ_i , it is clear that the electron energy spectrum falls off rapidly at energies not far below the energy of the electrons radiating at 2,695 MHz. This requires that the acceleration mechanism produces copious electrons with Lorentz factors between 100 and 1,000, but few with Lorentz factors much below 100. This will be discussed later.

Average energy per nucleon

An important parameter for the dynamics of variable sources is the average energy per nucleon, $\Gamma m_H c^2$, where m_H is the mass of the hydrogen atom. A lower limit on Γ occurs when there is no non-relativistic plasma present. In this case

$$\Gamma = \frac{\int_{E_i}^{E_j} EN(E) dE}{n_{rel} m_H c^2} \quad (11)$$

where k is the ratio of the energy in the protons to the energy in the electrons. Values of k in the literature range from 0 to $m_H/m \sim 1,836$ depending on the acceleration mechanism under consideration. As an example, with a power law energy spectrum with $p = 1$, and using the limits on γ_i and γ_j in Table 2,

$$\Gamma = (1+k) \frac{m}{m_H} \cdot \frac{\gamma_j}{\ln(\gamma_i/\gamma_j)} \sim 0.2(1+k) \frac{\gamma_j}{\gamma_i} \left(\frac{T_b}{10^{12}} \right) \quad (12)$$

Hence, unless $k < 5$ and $\gamma_j < 5\gamma_i$, $\Gamma > 1$ and the material inside a variable radio source is highly relativistic. For larger values of p the conditions are less stringent, but it seems likely that, in general, variable radio sources may contain purely relativistic plasma and that the internal sound speed is close to $c/3^{1/2}$.

This result can be used to argue against certain simple models of variable radio sources based on non-cosmological redshifts. In attempting to understand the apparent 'faster than light' expansion of some sources observed using VLBI techniques, it is sometimes suggested that the radio sources may be closer than is indicated by their redshifts⁹. The double sources that are observed would then be separating at velocities comfortably less than the speed of light, and might simply be similar to the well known giant double radio sources, but on a very much smaller scale.

The results found here, however, are independent of the distance to the sources. If each radio component of a double source expands freely, it will do so at about its internal sound speed, which we have shown to be very probably close to the speed of light. If the two components are separating more slowly than this then they will merge together, and a double source will not be seen at all.

Therefore, as in the case of the giant double radio sources, it is necessary to restrict the velocity of expansion to less than the velocity of separation. There are two obvious ways of doing this. First, one might add non-relativistic matter to reduce the internal sound speed. From the arguments already presented, this can be ruled out. Second, the components may be confined by the ram pressure of the external medium¹⁰. This requires $\rho_{ext} v^2 \sim$ internal energy density $\sim \Gamma c^2 \rho_{int}$, where ρ_{ext} and ρ_{int} are the external and internal densities, and v is the velocity of the component through the surrounding medium. But in this model, $\rho_{ext}/\rho_{int} \sim$ component size/component separation < 1 . Hence we require $v^2 > \Gamma c^2$, which contradicts the original assumption that the motion is non-relativistic.

Table 2 Lower limits on the low energy cut off, γ_i , of the electron energy spectrum

T_b (K)	p	γ_n	γ_i	γ_n/γ_i
10^{12}	1.0	1,000	> 41	< 24
	1.5	1,000	> 100	< 10
	2.0	1,000	> 161	< 6
10^{11}	1.0	100	> 10	< 10
	1.5	100	> 21	< 5
	2.0	100	> 30	< 4

More complicated schemes could doubtless be constructed, but the internal energy densities in these sources are enormous, regardless of their distance. It is, therefore, not very surprising if relativistic motions are observed. If the sources are at smaller distances than indicated by their redshifts, so that the measured motions are non-relativistic, then there may be severe difficulties in constructing models that are consistent with the observed limits on Faraday rotation.

Discussion

We have used the observed limits on Faraday rotation in variable radio sources to argue that (1) the density of any cold electrons present is very much less than the density of relativistic electrons; (2) the energy spectrum of the relativistic electrons cuts off rapidly at Lorentz factors not far below 100, and (3) the average energy per nucleon may well be $> m_H c^2$.

Further information concerning the electron energy spectrum can be obtained from the radio spectra of the outbursts. The rapid rate of decay of individual outbursts, and the relative rates of decay observed simultaneously at 2,695 and 8,085 MHz suggests that the radio spectrum steepens with time³. This would be consistent with a high-energy break in the electron energy spectrum that sweeps through the radio window as the source expands. More direct evidence is given by Andrew *et al.*¹¹ in the case of the giant 1968-69 outburst of 3C354.3. Here the spectrum of the outburst itself can be followed with some confidence, and again it is found that at late times the spectrum steepens drastically.

This high-energy cut off may be caused by radiation losses or may be intrinsic to the electron energy spectrum. But in any case, it seems that the electron energy spectrum is relatively narrow, extending neither to very high energies nor to very low energies. This is an important clue to the nature of the acceleration mechanism.

One possible spectrum of this type is a relativistic Maxwellian, of the form

$$N(E) dE = N_0 E^2 e^{-E/kT} dE \quad (13)$$

Formulae for computing the radiation from a relativistic Maxwellian distribution are given by Pacholczyk⁵. Such a distribution can arise naturally in a plasma at very high temperatures which is in thermodynamic equilibrium. The observed brightness temperatures suggest that plasma temperatures of the order of 10^{12} K are required.

One way of attaining such a high temperature has been discussed in some detail by Blandford and McKee¹². This involves a very high energy shock wave propagating through a thermal plasma at relativistic velocities. The jump conditions imply that in a co-moving frame just behind the shock, the mean Lorentz factor of the nucleons, $\Gamma \sim$ shock Lorentz factor. If the VLBI observations are interpreted in terms of relativistic kinematic effects¹³, then Γ is typically in the range of 1 to 10. If a fraction ϵ of the energy of the shocked particles goes into the electrons, then the electron temperature is

$$T \sim \frac{\epsilon \Gamma m_H c^2}{3k} \sim 3.6 \times 10^{12} \epsilon \Gamma \quad (14)$$

The appropriate value of ϵ is extremely uncertain, but if it is not too small, then electron temperatures of the order of 10^{12} K

can be achieved, and the electron energy spectrum may be close to a relativistic Maxwellian. If at any time the electron temperature is much greater than 10^{12} K, then 'inverse Compton cooling' can rapidly pull the temperature down to 10^{12} K again¹⁴. The radiation from a relativistic blast wave is discussed by Blandford and McKee¹⁵, who consider both power law and relativistic Maxwellian electron energy spectra.

The amount of Faraday rotation expected from a relativistic Maxwellian distribution can be computed easily; from Sazonov (1969), the equivalent density of cold electrons is

$$n_c = - \int_0^\infty \gamma \ln \gamma \frac{\delta}{\delta \gamma} \left[\frac{1}{\gamma^2} N(\gamma) \right] d\gamma \quad (15)$$

Inserting equation (13), and writing $\gamma_0 = \frac{kT}{mc^2}$

$$n_c \sim \frac{n_{rel}}{2\gamma_0^2} (\ln \gamma_0 + 1 - \xi) \quad (16)$$

where ξ is Euler's constant, and $\gamma_0 \gg 1$.

If we are observing in the optically thin part of the spectrum, but below the peak, that is $\gamma = (v/v_B)^{1/2} < \gamma_0$, then we find

$$\chi = \frac{9.3^{1/3}}{4\pi} \left(\frac{\gamma}{\gamma_0} \right)^{-2/3} \frac{(\ln \gamma_0 + 1 - \xi) T_b}{\gamma_0 T} \cot \theta \quad (17)$$

$$\approx \frac{1}{\gamma_0} \ll 1 \text{ if } T \sim T_b \sim 10^{12} \text{ K}$$

Conclusions

We have shown that the observed lack of Faraday rotation in variable radio sources places severe restriction on the density

of both thermal plasma and low energy relativistic electrons. The fact that $n_{rel}/n_c \gg 1$ can be taken as direct evidence against all models in which the electrons are accelerated by betatron and Fermi processes in a turbulent plasma¹⁶.

It is also clear that the relativistic electron energy distribution does not extend to very low energies. There is weaker evidence from the radio spectra that the distribution does not extend to very high energies either, and may in fact be relatively narrow.

One possible distribution function of this form is a relativistic Maxwellian, such as may be produced behind a relativistic blast wave¹⁵. This automatically gives negligible internal Faraday rotation and if the VLBI observations are interpreted as relativistic kinematic effects, naturally leads to brightness temperatures of 10^{11} – 10^{12} K. The amount of internal Faraday rotation implied by equation (17) is so small, that it may be necessary to consider the decreasing Faraday rotation external to the shock, caused by sweeping up the ambient medium. In some situations this can produce the dominant contribution to $\Delta\chi$.

In a future paper we shall show that relativistic blast wave models can account for several other features of the spectra, flux variations and polarisation of variable radio sources.

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Uranium anomaly in Black Sea sediments

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The upper 90 cm of Black Sea basin sediment with an areal extension of $2.96 \times 10^5 \text{ km}^2$ has an U_3O_8 content of 6.7×10^6 tonnes. Plankton is the prime agent for uranium fixation. Reducing conditions at depth permit uranium to accumulate over the past 5,000 yr. Energetically self-sufficient burning of the top 1-m strata will lead to U_3O_8 concentrations in the order of 100 g per tonne ash.

THE Black Sea is the largest anaerobic water body in the world ocean and measures almost half a million km^3 (ref. 1). Its present environmental state is a consequence of Holocene sea-level rise and associated formation of a stable pycnocline restricting free exchange of molecular oxygen to the deep water². Holocene sediment cores from the euxine abyssal plain show, almost in slow motion, the development from a fully oxygenated fresh water 'Black Lake' to the modern brackish-marine Black Sea³. A typical 1 m sediment section consists from top to bottom of: coccolith ooze, sapropel, and light lutite. The sapropel-lutite boundary, with an assigned age of about 5,000 yr BP

marks the event when the Black Sea became permanently stratified and euxinic conditions established at the sediment-water interface⁴.

Fluctuations in redox potential may cause depletion or enrichment in certain elements both for water and sediment. Here we report on the mechanism of uranium concentration in Black Sea sediment as a function of a changing habitat.

Samples and analytical procedures

The uranium content in abyssal Black Sea mud is almost one order of magnitude larger than in average marine sediment^{5, 6}. Similar concentrations are known from sediments of Norwegian fjords and the Baltic Sea^{7, 8}. It seems, therefore, that restricted environments with a trend to euxinic conditions favour accumulation of uranium.

In the Black Sea abyssal mud the U_3O_8 concentration rarely exceeds 50 p.p.m. and 25 p.p.m. can be taken as a representative mean value. In spite of the high uranium level and the wide areal distribution, the anomaly seems to be, on first sight, of no economic significance. This outlook, however, may change by closer examination of the deposit from a sedimentological and geochemical point of view. This is because the high content

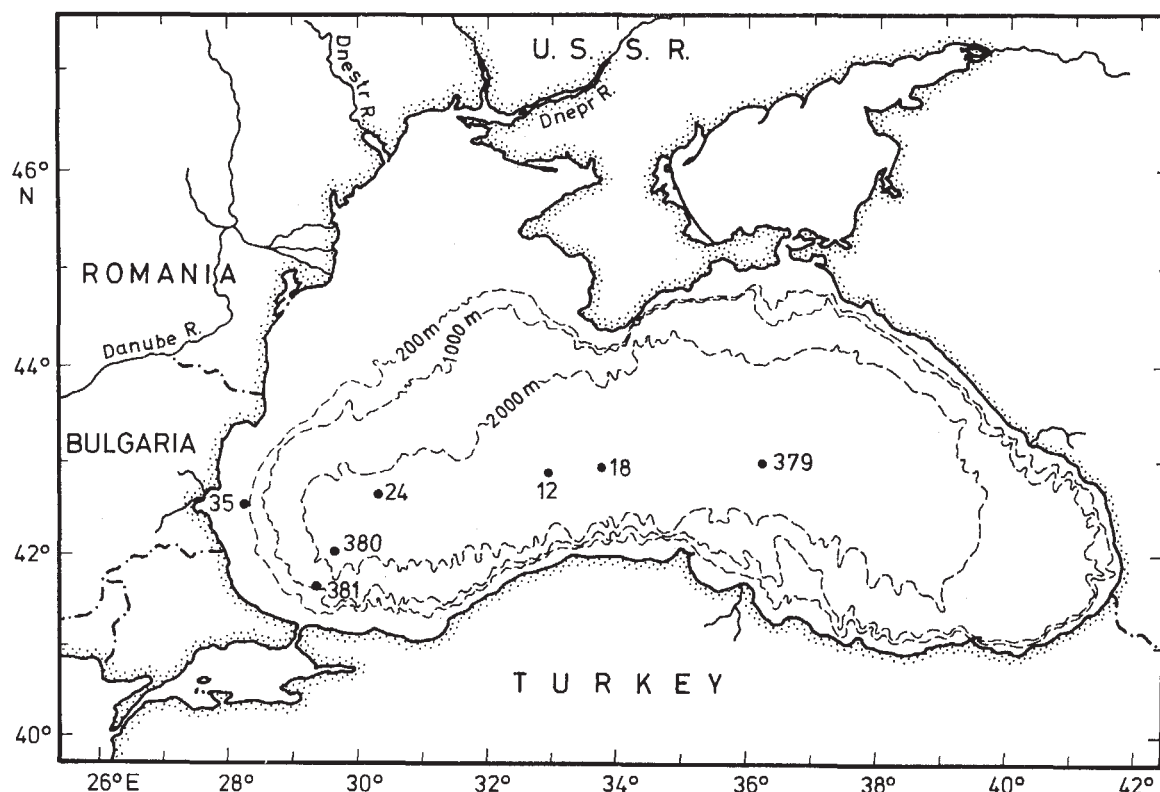


Fig. 1 Bathymetric chart of Black Sea and location of stations.

of organic matter and carbonate may allow for a considerable reduction in sediment mass by burning; furthermore, the material is unconsolidated and thixotropic.

Water and sediment were collected during RV Chain cruise of the Woods Hole Oceanographic Institution in Spring 1975. Location of stations is shown in Fig. 1. The sample collection has been supplemented with Pleistocene sediments obtained during the deep-sea drilling operation of Glomar Challenger, Leg 42 in the Black Sea in May–June 1975 (Fig. 1).

Uranium determinations were carried out spectrophotometrically using methods previously developed^{9,10}. Principally, uranium was extracted with tri-*n*-octylphosphine oxide (TOPO); and 2-(5-bromo-2-pyridylazo)-5-diethyl-aminophenol (bromo-PADAP) was used as a chromogenic reagent. Uranium nitrate was used as spike and syenite rock SY-2 and SY-3 from Canadian certified reference materials project, Mines Branch, Ottawa, were used for intercalibration.

Results

The three stratigraphic units—coccolith, sapropel and lutite—can be distinguished by their carbonate and organic matter content (Table 1). The core section is close to Station 379 (Fig. 1). The carbonate fraction in the lutite unit is principally composed of reworked Cretaceous and Tertiary coccoliths, whereas in the sapropel and coccolith units it is authigenic. The organic matter in the sapropel is principally land-derived, particularly at the base of the unit. Increase in carbonate content towards the top signals a progressively higher input of marine planktonic material.

The U_3O_8 content in the sediment matches closely this stratigraphic development. The freshwater lutite unit deposited in aerobic conditions shows the smallest enrichment, and the brackish-marine coccolith ooze at the bottom of the euxinic abyssal plain the highest one (Table 2; core 18). 'Dilution' of coccolith ooze by detrital clay minerals going from the central basin towards the Bulgarian coast, that is from Station 18 to 35, is reflected in a lowering of the U_3O_8 content in the coccolith ooze (Table 2). On the basis of a few analyses from core material

drilled by Glomar Challenger a similar trend seems to be established. A calcareous mud (Table 2; 379 A) deposited in aerobic conditions has little uranium, whereas the five remaining samples formed in an anaerobic environment show a 10–20-fold enrichment in U_3O_8 . The highest values again are found in the two samples from the central basin (Station 379 A).

Treatment of sample material with HCl or water, or by heat combustion, may cause uranium depletion or enrichment in the

Table 1 The carbonate and organic matter content of the stratigraphic units

Depth (cm)	Unit	CaCO ₃ (%)	Organic C (%)	Organic N (%)
2	Coccolith	41.2	2.86	0.25
5		56.2	3.84	0.33
8		34.9	3.53	0.31
12		60.7	4.31	0.37
15		65.7	5.10	0.44
18		48.2	5.17	0.44
22		14.9	5.91	0.49
25	Sapropel	16.5	7.17	0.60
28		11.0	11.45	0.95
32		16.5	12.23	1.11
35		12.4	13.45	1.18
38		7.8	14.35	1.24
42		8.9	15.73	1.26
45		6.9	14.10	1.26
48		6.9	16.85	1.38
52		3.4	19.90	1.41
55		5.0	18.65	1.37
58	Lutite	3.4	17.42	1.15
62		3.8	15.35	1.02
65		4.3	15.50	1.01
68		12.6	4.70	0.39
72		6.0	2.07	0.19
75		10.2	0.31	0.030
78		10.7	0.45	0.032
82		1.8	2.60	0.24
85		0.54	1.60	0.17
88		9.4	0.81	0.09
92		8.8	0.75	0.07

Table 2 The U_3O_8 content of the stratigraphic units

Core	Sample	Depth	Wt-loss (%) 0–100 °C	Wt-loss (%) 0–1,000 °C	(1)	(2)	U_3O_8 (p.p.m.) (3)	(4)	(5)
18	Coccolith ooze	4 cm	68.7	80.5	55.0	113.9	55.4	73.9	94.3
	Coccolith ooze	21 cm	66.0	77.6	59.7	34.6	9.0	74.7	47.2
	Sapropel (top)	30 cm	65.5	74.6	23.4	17.3	40.4	86.4	17.3
	Sapropel (base)	85 cm	71.0	81.9	15.7	40.1		110.0	55.1
	Lutite	100 cm			2.4				
12	Coccolith ooze	10 cm	61.9		35.4				
24	Coccolith ooze	4 cm	66.5		28.3				
35	Coccolith ooze	30 cm	58.4	68.5	15.0			24.3	
	Coccolith ooze	100 m	38.5		40.1		118.3		
379 A	Sapropel	100 m	24.6		49.5		95.8		
	Calcareous mud	231 m	35.3		2.4				
380 A	Carbonaceous lutite	673 m	25.6		35.4				
	Diatomaceous marl	837 m	30.5		20.4				
381	Diatomaceous mud	237 m	37.0		33.8				

(1) After drying at 110 °C; (2) after drying at 110 °C and treated with 10% cold HCl and washed with H_2O ; (3) after drying at 110 °C and hydrolysed with 6 M HCl for 22 h and washed with H_2O ; (4) after ignition at 1,000 °C; (5) after ignition at 1,000 °C and washed with H_2O .

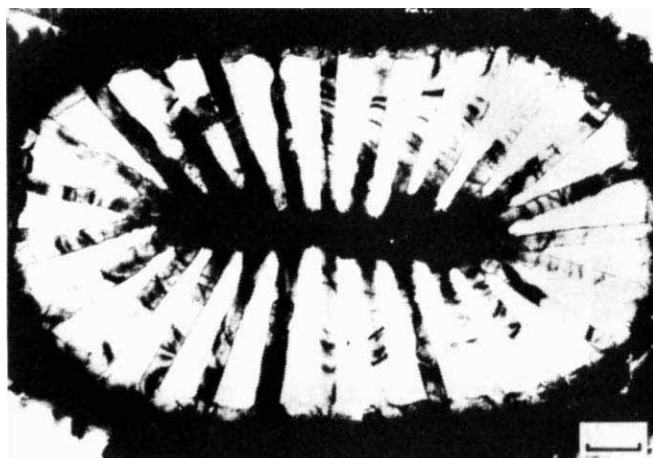
residue (Table 2). Of special significance is the high U_3O_8 yield of the mineral ash obtained in the combustion experiment, and the release of uranium by extracting the sapropel ash with water.

U_3O_8 concentration diminishes because of: (1) loss of mineral-bound water; (2) loss of sulphur-containing volatiles, (3) loss of salts, (4) calcining of carbonates, and (5) burning of organic matter. U_3O_8 depletion is caused by: acidification, $\text{CaO-H}_2\text{O}$ interactions, and release of water-soluble uranium-organic complexes.

The bulk of the uranium seems to be bound to planktonic matter; land-derived organic debris contains comparatively little uranium. Coccoliths seem to be the prime host for uranium in modern Black Sea sediments, but other planktonic organisms share this affinity. The following tentative model is suggested to account for the enrichment.

Eukaryotic cells contain complex membraneous organelles known as the Golgi apparatus or Golgi body which occupy a central position in the transport system of the cell. For example, they fix and transport metals from within the cell to the outer membrane. This is accomplished by metal-ion coordination to specific proteins and polysaccharides which are subsequently transported to the outside. Biomineralisation is an outgrowth of this process¹¹. In the case of coccoliths, uronic acids and polysaccharide-sulphates¹² are the principal metal-ion fixers. The relationships are revealed in an electron micrograph where the organic template is stained by heavy metals, thus revealing the growth pattern of the coccolith within the Golgi body. In this way, metal ions that are not needed by the organism can be readily neutralised (Fig. 2).

Fig. 2 Heavy-metal stained coccolith, *Umbilicosphaera* sp., revealing growth pattern. Scale bar, 0.2 μm .



Uranium content of Black Sea water ranges from 1 to 7 p.p.b., (parts in 10^9) with the average ~ 3 p.p.b. (refs 13 and 14) which is about the value for standard mean ocean water. Our U_3O_8 values are within the same order of magnitude: aerobic zone (2.4 p.p.b.), interface (5.9 p.p.b.) and anaerobic zone (3.5 p.p.b.). Assuming that coccoliths fix the uranium principally during the life cycle of the organism, a 10,000-fold enrichment is observed.

Marine calcareous material of biological origin is reported to contain at most a few p.p.m. uranium¹⁵. In general, carbonates are less favourable host materials for uranium¹⁶. Thus, the enrichment of U_3O_8 in the Black Sea coccolith unit is unexpected and we are not aware of any systematic study on uranium in coccoliths. In view of the nature of the calcifying matrix in coccoliths, however, we tentatively conclude that the uronic acids and sulphated polysaccharides act as a substrate in the formation of hexavalent uranium complexes.

Non-calcareous plants may operate in the same fashion if their cell walls contain such sugars as they do in diatoms. Studies on a Holocene lake in central Ontario, for example, which is polluted by discharge from a uranium mine shows a 10,000-fold U-enrichment in the diatom-dominated living plankton over the water, that is 210 p.p.m. against 20 p.p.b. The sediments, at a water depth of 10 to 25 m, are principally diatomite and reducing in character; their U-content is between 170 and 380 p.p.m. It thus seems that reducing conditions in the depositional environment are only essential for the preservation of uranium-enriched detritus, and not for the fixation. Following sedimentation, a series of organic molecules may pick up additional increments of uranium as well as other heavy metals from sediment and water. The organic material can become stabilised by heavy-metal complexation to a point that it is finally rendered insoluble to extraction by conventional acid or base treatments.

Mass balance

The Black Sea covers an area of $4.23 \times 10^5 \text{ km}^2$, of which 30% is shelf. The basin proper, therefore, has an areal extent of $2.96 \times 10^5 \text{ km}^2$. The top 1 m sediment has a bulk density of 1.25 g cm^{-3} , which will give a sediment mass of $3.7 \times 10^{17} \text{ g}$. Combustion of total sediment at 1,000 °C will reduce weight of material by 80%; weight of remaining ash is $7.4 \times 10^{16} \text{ g}$. Average U_3O_8 content in ash (Table 2) is $90 \times 10^{-6} \text{ g per g}$ sediment. Total U_3O_8 concentration in sediment ash of top 1 m strata of Black Sea basin is $6.7 \times 10^{12} \text{ g}$ or 6.7×10^6 tonnes.

Sapropel and coccolith ooze have been deposited over the past 5,000 yr. Assuming that uranium is extracted at a constant rate over this time, the basin sediments will annually receive $1.34 \times 10^9 \text{ g U}_3\text{O}_8$. Should this material exclusively be extracted from the aerobic water layer having a mean U_3O_8 content of $3 \times 10^{-6} \text{ g l}^{-1}$ or a total of $1.78 \times 10^{11} \text{ g U}_3\text{O}_8$ for the upper 200 m, about 1% of this amount is annually released to the sediment.

Calorimetric determination of combustion heat

Average Black Sea abyssal mud deposited over the past 5,000 yr contains per 1,000 g sample: 600 g H_2O , 100 g clay, 100 g organic matter, and 200 g CaCO_3 . Since combustion of sediment at 1,000 °C will substantially increase the U_3O_8 content in the remaining ash, it is of considerable interest to know whether indigenous organic matter can supply sufficient energy for this reaction.

Calcining of carbonates: $\text{CaCO}_3 \rightarrow \text{CaO} + \text{CO}_2$ $E = 47.2$ kcal mol^{-1} and evaporation of water: $\text{H}_2\text{O}(\text{aq}) \rightarrow \text{H}_2\text{O}(\text{g})$ $E = 9.73$ kcal mol^{-1} require a total of 418 kcal per 1,000 g of sediment. This is the minimum amount of energy needed for self-sufficient burning.

Table 3 Combustion heat values for the stratigraphic units compared with common organic compounds

Sample	Combustion heat (Kcal kg^{-1} at 25 °C)
Coccolith*	528
Top sapropel*	642
Bottom sapropel*	1,109
Wood†	4,500–4,800
Peat†	5,000–5,800
Lignite†	6,200–7,600
Coal†	7,600–8,750

*Samples were pre-dried at 110 °C.

†See (ref. 18).

Combustion heat was determined for three representative samples from Station 18 (Fig. 1), coccolith ooze, top sapropel, and bottom sapropel. A conventional calorimetric bomb was used and combustion heat values are given in kcal kg^{-1} at 25 °C. Data are reported in Table 3 together with calorimetric values of common organic compounds.

Combustion heat values for the dry mud samples are considered minimum values, because part of the heat generated

during the experiment has been used for calcining, thermal degradation of clay minerals, and decomposition of sulphides. Still, these values are far in excess of the 518 kcal needed to dry and calcine a 1,000 g wet sediment.

In conclusion, heat combustion of Recent Black Sea mud will release more energy than is required for drying and thermal degradation of mineral matter. The combustion process will yield an ash with a U_3O_8 content close to 100 g tonne^{-1} . Slightly higher values are expected in those parts of the Black Sea basin which are furthest removed from river outlets and turbidite incidents. Such a region could be close to Station 379 (Fig. 1).

Although U_3O_8 values of 100 g tonne^{-1} are not economically significant now, the situation may change in the years to come should global demand for uranium expand at predicted rates. In view of the high sulphur content of recent Black Sea mud a note of caution should be sounded, since mining of the low-grade ore may introduce environmental hazards.

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Ozone concentrations in South-East England during the summer of 1976

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Surface ozone concentrations at five sites in South-East England were measured during the heat wave of 1976 from 22 June to 12 July. At all sites peak hourly concentrations were the highest ever recorded in the UK and the elevated levels persisted for periods longer than any previously measured. Some discussion of the significance of the results is given together with an analysis of back-track air mass trajectories in the context of oxidant formation in North-West Europe.

We have previously reported measurements of atmospheric ozone in urban and rural areas of southern England during the period 1971–75 (refs 1–5). Although, on occasion, the levels exceeded the US (US EPA) air quality standard of 80 p.p.b. (volume parts per 10^9 , $\sim 160 \mu\text{g m}^{-3}$) maximum 1-h mean⁶, the World Health Organisation (WHO) long-term goal of 60 p.p.b. $120 \mu\text{g m}^{-3}$ (ref. 7) and the Greater London Council (GLC) guideline of 80 p.p.b. ($160 \mu\text{g m}^{-3}$) (ref. 8), the occurrence of elevated levels was, in most instances, confined to irregularly spaced episodes of only a few days duration. During 1976, the unusual meteorological conditions prevailing between 22 June and 12 July

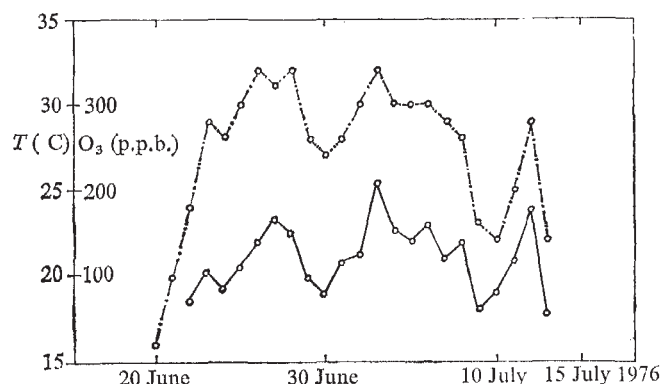


Fig. 1 Ozone and temperature levels during the photochemical episode. $\circ$ — $\circ$ — $\circ$, temperature at noon, mean of readings at Kew, Gatwick and Heathrow ($^{\circ}\text{C}$). $\circ$ — $\circ$, highest hourly mean ozone concentration for each day at Stevenage, p.p.b. (parts in 10^9).

resulted in a period particularly favourable for photochemical production of ozone. We present here data showing that the ozone concentrations at all our measuring sites were both higher and more persistent than any we have monitored previously. To illustrate the broad features of the persistence of elevated levels in South-East England during this period we have plotted, in Fig. 1, the highest hourly mean ozone concentration for each day at one of our sites (Stevenage) against the temperature at noon (mean of readings at Kew, Gatwick and Heathrow).

Ozone concentrations are reported from five locations in South-East England, three within the Greater London conurbation and two outside in rural (Harwell) and semi-rural (Stevenage) environments. The Greater London sites were carefully chosen to minimise the effect of local sources of nitric oxide (NO) which is initially a rapid scavenger of ozone. In the centre of London, where this is difficult to achieve, a rooftop site overlooking a courtyard was chosen (Islington, on the City boundary). The suburban sites (Hainault and Teddington) were in mixed open parkland/residential locations. The Warren Spring Laboratory site lies on the western edge of Stevenage New Town with open country to the south and west. Two local sources of NO are a light industrial area to the north and the A1(M) motorway some 40 m to the west. The only significant sources of NO at the Harwell site are likely to be traffic on the A34 road ~ 1.5 km to the east and vehicles on the AERE site itself. The locations of the sites are shown in Fig. 2, and specific site details in Table 1.

Three sites were equipped with ethene-chemiluminescence⁹ (EC) ozone monitors and two with instruments operating on the ultraviolet absorption principle¹⁰ (UV).

All instruments were calibrated using the neutral KI method¹¹. In addition, one instrument was calibrated using the optical extinction coefficient at 255 nm (ref. 12) in a 1.2-m absorption cell, and two others by gas-phase titration based on the $\text{NO} + \text{O}_3$ reaction. From a consideration of the experimental errors involved in calibration and the systematic errors in sampling and data collection, we estimate the hourly mean data bounded by 2σ confidence limits of $\pm 10\%$ of the stated values.

The results obtained from the five instruments are shown in Fig. 3. There are differences in detail but the overall similarity between the results measured at the different sites is striking.

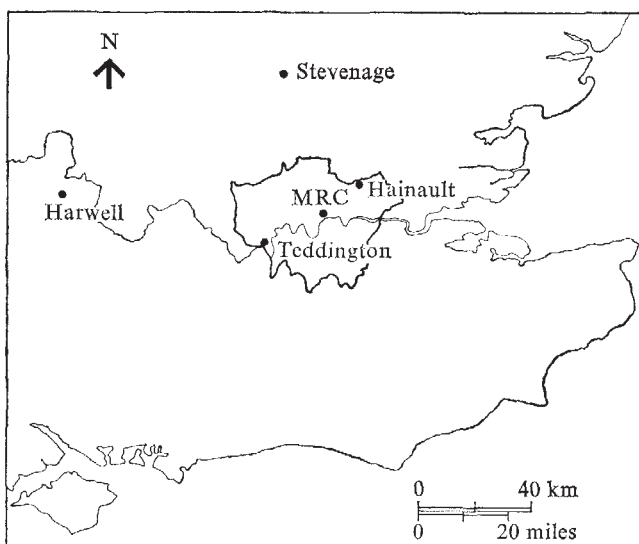


Fig. 2 Sketch map of South-East England showing location of sampling sites.

We have attempted to quantify the similarities and differences between the sites by investigating the correlations between the hourly mean ozone concentrations for each pairing of sites and the results are shown in Table 2. Initially, hourly values from one site were regressed against the concentrations for the same hours at each of the other sites in turn. The results in Table 2 show, that, in general, the Harwell ozone levels are higher than the other sites for which no general pattern can be distinguished. We then investigated, for pairs of sites, the correlations of hourly values displaced relative to one another, that is hour n for site A was correlated with hour $n+m$ of site B. This was done for all pairs of sites for values of m from -11 to $+11$. It was found that for all pairs of sites not involving Harwell,

Table 1 Site details

Site	Site description	Height above ground (m)	Instrument	Calibration
City (C)	Courtyard in centre of Greater London ~ 70 m from nearest street	20	EC	KI
Hainault (Ha)	Suburban London. Open country to S and E, otherwise residential	4	UV	KI + gas phase titration
Harwell (H)	Rural, 1.5 km from A34 trunk road	2	EC	KI + optical extinction
Stevenage (S)	Light industry to N, motorway 40 m to W, residential to E, otherwise open country	10	EC	KI
Teddington (T)	Suburban London. Open parkland to S, residential to N	13	UV	KI + gas phase titration

Table 2 Inter-site correlations of ozone levels for 1976 episode

Site versus Site		Lag* (h)	Correlation coefficient	Mean regression†	Significance level of correlation coefficient change with lag (%)
H	S	0	0.78	$S = 0.77H - 7.9$	
H	C	0	0.74	$C = 0.72H - 13.2$	
H	T	0	0.74	$T = 0.72H - 7.7$	
H	Ha	0	0.67	$Ha = 0.73H - 11.3$	
H+1	S	1	0.80	$S = 0.77H - 7.8$	79
H+1	C	1	0.76	$C = 0.71H - 12.7$	77
H+1	T	1	0.81	$T = 0.72H - 7.4$	99
H+2	T	2	0.81	$T = 0.72H - 7.5$	99
H+1	Ha	1	0.72	$Ha = 0.73H - 10.5$	91
H+2	Ha	2	0.73	$Ha = 0.73H - 10.4$	93
S	C	0	0.78	$C = 0.93S - 5.5$	
S	T	0	0.86	$T = 0.94S - 1.5$	
S	Ha	0	0.81	$Ha = 0.94S - 2.8$	
C	T	0	0.81	$T = 1.0C + 5.5$	
C	Ha	0	0.88	$Ha = 1.0C + 2.7$	
T	Ha	0	0.81	$Ha = 1.0T - 2.8$	

H, Harwell; S, Stevenage; C, City; T, Teddington; Ha, Hainault.

*For example, H+1 means hour $n+1$ for Harwell was correlated with hour n for other sites.

†Mean of y on x and x on y .

the correlation coefficient for $m=0$ was highest. In contrast, for correlations of Harwell values against those from all other sites, improved correlation coefficients were obtained with $m = +1$ or $+2$, that is, better correlations were obtained by regressing hourly values from the other sites against later hourly values at Harwell. Two points emerge clearly from this analysis; the results for the Stevenage site are similar in magnitude and diurnal pattern to the Greater London sites and Harwell is significantly different from each of the other sites in both respects. The explanation of both points is in part linked with local emissions of NO and the close proximity of the light industrial area and motorway turning the otherwise rural aspect of the Steven-

age site into a more urban situation while the relatively low NO levels at Harwell lead to less ozone depletion. The important conclusion is that, in common with the Los Angeles area¹⁹ it is the rural areas downwind of the primary pollutant sources which experience the higher ozone levels.

The maximum 1-h mean concentrations of ozone measured at each of the sites exceeded those previously reported by considerable margins (see Table 3).

During the 21-d period 22 June to 12 July the numbers of hours above 100 p.p.b. within Greater London were 85, 86 and 100 at the City, Hainault and Teddington sites and 115 and 158 at Stevenage and Harwell, outside Greater London. The total number of hours above 100 p.p.b.

Fig. 3 Ozone concentrations in p.p.b. (y axis) measured at each site during the photochemical episode. H, Harwell; T, Teddington; Ha, Hainault; C, City; S, Stevenage. *a*, 22 June; *b*, 29 June; *c*, 6 July.

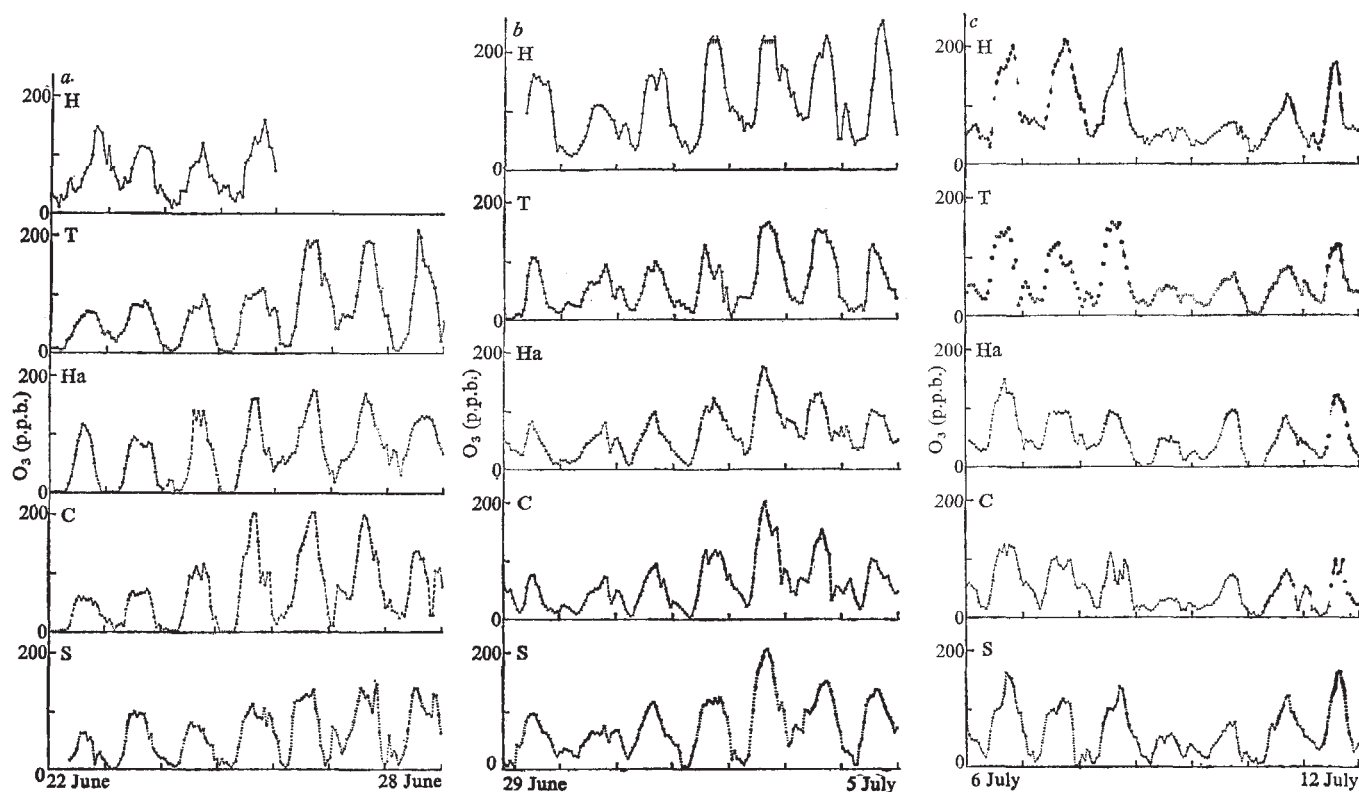


Table 3 Highest hourly mean ozone concentrations observed during 1976 compared with previous measurements

Location	Year	Highest hourly mean, p.p.b.	Ref.
Central London	1972	112	2
	1973	136	3
	1974	164	13
	1975	150	4
	1976	210	This work
London suburbs	1975	130	4
	1976	210	This work
	1976	210	This work
Rural southern England	1971	101	1
	1972	128	14
	1973	141	3
	1974	120	12
	1975	177	12
	1976	258	This work

accumulated during the whole period 1971–75 at the Harwell site was exceeded in this one 1976 episode. For the first time in the UK, urban levels exceeding 200 p.p.b. and rural levels exceeding 250 p.p.b. were measured. At each site there was a continuous period of 5 d or more (Table 4) when the ozone concentration exceeded 100 p.p.b. for at least 8 h each day. Thus the industrial threshold limit value (TLV)¹⁵ was exceeded at all our sites in the outside air in South-East England for the equivalent of a working week. In this analysis the duration of high concentrations at Stevenage was intermediate between that in the urban and rural extremes of London and Harwell. Although these levels were high in comparison with the air quality guides and industrial threshold limit values cited above, it is not possible to evaluate their direct significance in terms of human health. Comparison with the TLV is not necessarily appropriate for such limiting values are intended for use in evaluating health risks for the occupationally exposed. For the general population, which includes the young and the infirm, a further margin of safety is usually regarded as prudent.

The US air quality standard and the WHO long term objective are more stringent than the TLV. Both were exceeded during 1976; but, they are also exceeded in the US and in many other developed nations. There was a sharp increase in mortality in Greater London at the time of this episode¹⁸ but effects due to anything other than the high prevailing temperatures could not be isolated. During early July serious damage was observed on two crops of tobacco plant (Bel-W3) grown specifically to test for oxidant effects at Leeds and at the Fairfield Experimental Horticulture Station, Kirkham, Lancashire (J. T. Fletcher, personal communication). High percentage leaf damage would be anticipated on the basis of models of tobacco plant injury¹⁶ and the ozone concentration reported here. No effects of elevated oxidant levels to any other types of plant were reported during the episode.

Elevated ozone levels in the UK have generally been associated with anticyclones to the east of the UK³ and to this extent the photochemical episode of 22 June to 12 July was typical. In such cases the air mass trajectories over

southern England are easterly and in general cross NW Europe. Typical back-track trajectories are illustrated in Fig. 4. Those for 27 June and 3 July describe the history of the air mass reaching South-East England on two of the days of particularly high ozone concentrations. On 7 July the levels at most sites had begun to decrease and 9 July, the trajectory for which shows a south-westerly air flow over South-East England, was the day on which all sites showed their lowest daily maximum hourly mean ozone concentration throughout the episode.

A closer study of individual back-track trajectories highlights a number of factors which must be borne in mind

**Fig. 4** Back-track air mass trajectories for four selected days during the photochemical episode. The location of the air mass at various times in hours before its arrival at midday in London is indicated on each trajectory. *a*, 27 June; *b*, 3 July; *c*, 7 July; *d*, 9 July 1976.

if an abatement policy for photochemical oxidants were to be considered. First, generation of ozone requires several hours of solar irradiation of the primary pollutant plume even with the more reactive hydrocarbon precursors so that photochemical ozone is usually first detected 50 km or more downwind of the primary source¹⁷. In the densely populated and highly industrialised regions of North-West Europe this makes source identification difficult. Second, photochemical ozone can be transported over hundreds of km from source regions in North-West Europe with the limited deposition at sea surfaces and because ground-based nighttime inversions typical of anticyclonic weather isolate the ozone formed during the day from ground sink surfaces over rural areas³. Third, primary NO emissions, while constituting an important precursor of photochemical ozone are initially a potent sink for ozone in more polluted areas and the emission and dispersion pattern of NO will have a major effect on ozone concentrations measured close to the ground. These factors tend to obscure any simple interpretation of the effect of a particular precursor source region.

As an example, we consider here the trajectory of 3 July (*b*, in Fig. 4). The air mass present over South-East England on 3 July had travelled across southern Poland, a possible

Table 4 Maximum number of consecutive days with ozone > 100 p.p.b. for at least 8 h

Location	No. of days	Longest period
City	5	24 June–28 June
Hainault	5	24 June–28 June
Teddington	6	3 July–8 July
Stevenage	10	1 July–9 July
Harwell	18	22 June–9 July

area of precursor emission, about 45 h previously. Then there was about $\frac{1}{8}$ cloud cover reducing the radiation flux and a temperature of about 25 °C so that photochemical activity was probably limited. Throughout the afternoon of 2 July the air mass traversed central West Germany with clear sky conditions and temperatures of 30 °C. The conditions for precursor injection and photochemical transformation were potentially excellent. By the morning of 3 July the air had reached the industrial areas and refineries of the Thames estuary, also potential sources of precursors, with clear sky conditions still prevailing. At this time the air mass was moving at about 400 km d⁻¹, although surface winds were only 4–10 km h⁻¹. Thus several hours later the air reached central London where maximum ozone values were recorded during the period 1400–1600 BST. The air mass which gave rise to the elevated ozone levels on 3 July, therefore, had 3 d with probable precursor injection and two full days of exposure to strong sunlight and high temperatures during which the ozone was formed. The history of air over London on other days in this episode with high recorded ozone levels was similar.

It has previously been noted that the data for Greater London follow a pattern which is consistent with the mechanism of local generation of ozone, both from the point of view of precursor emissions and the concentration gradient of ozone across London from east to west^{2,4}. That proportion of the total ground level ozone concentration which is produced locally is difficult to estimate solely from ground-level measurements. The complexities of the air mass trajectories presented here and the likely emissions of precursors along their paths together with considerations of precursor reaction times and NO emissions all tend to merge local production into the general pattern of secondary pollutant formation in North-West Europe as a whole.

Our further understanding of this phenomenon and the

role of various primary pollutants clearly requires the co-operation of other European countries, upwind and downwind of the UK, in ozone and precursor measurement programmes. Data on the occurrence of high ozone concentrations in North-West Europe in the period 1971–75 have been assembled in a recent publication²⁰ and we would welcome the further publication and/or exchange of relevant data from those areas which the back-track trajectories indicate to be of importance in oxidant production.

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Palaeoclimates of Central Sahara during the early Holocene

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In the Central Sahara lying approximately between 27°N and 18°N, rains were primarily due to tropical depressions in the early Holocene up to about 6500 BP. Then the monsoon rains of Sahelian type dominated up to about 4400 BP.

POLLEN analysis was carried out on Holocene lacustrine deposits¹ sampled every 10–20 or 30 cm in a section of about 7.80 m at Tjéri (13°44'N–16°30'E) near the centre of the great Palaeochad^{2,3} (Fig. 1). Chronology of the Tjéri section was established by two radiocarbon dates on organic material near the base (Fig. 2) and, for some other levels, by correlations with notable events radiocarbon dated elsewhere in the zone of the Palaeochad^{1–3}. The chronology established in the Nile valley (see below) and elsewhere in the southern part of the Sahara gives some valuable correlations¹. Approximate dating of the intermediate levels was established by rates of sedimentation. About 60 yr elapsed for each 10 cm from 9000 to around 7000 BP and about 220 yr from then till about 4000 BP. The change in rates of sedimentation at about 7000 BP could parallel the important change in conditions noted for the Blue Nile at about 7000 BP (ref. 4).

In the dry tropical zone where the Chad basin is situated, the rainfall and its distribution through the year are the most important climatic factors controlling the vegetation and its spatial distribution. Thus there is a direct relationship between vegetation and climatic pattern. In the Chad basin the regular succession of climatic and vegetation zones is a favourable factor for pollen analysis⁵. On the Tjéri section 46 samples were studied and for each about 1,000 to 6,000 pollen grains were counted. The Gramineae, Cyperaceae and *Typha* pollen grains represent about 80 to 90% of each total. Therefore those 3 taxa have been eliminated from the pollen sum and studied apart in order not to distort the percentages of all other pollen grains more typical. Those pollen grains were classified according to the present geographic distribution of the taxa to which they belong and the most typical were placed in four phytogeographical elements covering the four major vegetation zones of the Chad basin, that is, (from S to N) Sudano-guinean element (plants typically growing under 1,500 to 1,000 mm rain per annum), Sudanian element (1,000 to 500 mm), Sahelian element (500 to 100 mm), Montane (Tibesti) element (plants growing at the nearest on the upper Tibesti Plateaux) and also in a group of hygrophilous plants⁵. The relative percentages of the pollen grains for these four elements were used to construct curves which portray the climatic variations occurring in the four zones of the Chad basin¹.

Palaeoclimatic pattern of the Sahelian zone

Comparison of the Sudano-Guinean element and Sahelian element curves showed that the periods of climatic optima (relatively wetter phases) are in general out of phase with each other¹ (Fig. 2). It seems also that during the Holocene, the Sahelian climatic optima have always been synchronous with the warming periods, and the deteriorations with the coolings. Indeed the trends of the Sahelian curve at Tjéri—the amplitudes of variations being different—correlate well with the trends which appear on some curves portraying the evolution of the temperature on the Northern Hemisphere, such as that of Camp Century in Greenland^{6,7}. For the most part of the Holocene, it seems that the Camp Century curve is quite well dated: first, the counting of annual layers was possible with some correction until 8300 BP (ref. 7); second, for the Holocene this curve has good cross-checkings with various eustatic curves^{8–10}, with the fluctuations in the

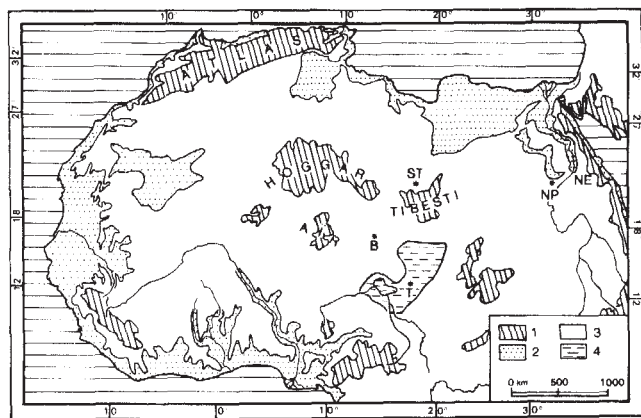


Fig. 1 North Africa. 1, Mountains over 1,000 m. 2, Regions under 200 m. 3, Regions between 1,000 m and 200 m. 4, Holocene Palaeochad, 320 m high. T, Tjéri. B, Bilma. ST, Sérir Tibesti. NP, Nabta Playa. NE, Egyptian Nubia.

atmospheric radiocarbon level^{11,12}, with glacier fluctuations in the Alps¹³, and so on. Comparison of the Sahelian curve with that of Camp Century shows that the lowering periods of the Sahelian curve, which correspond to aridification periods in the Sahel zone, correspond also to cooling periods, and vice versa¹. Study of diatoms^{2,14} and pollen in the same samples provides a direct and important corroboration of this phenomenon. Indeed, from about 8000 up to about 4000 BP, the high percentages of a psychrophile ('cold') diatom *Cymatopleura elliptica* or of a diatom of temperate type *Cyclotella ocellata* occur during the phases of relative lowering or minima of the Sahelian element (Fig. 2). Considering these different correlations, one can also use the curve of Camp Century as a basis for an explanation of the Montane element.

The Montane (Tibesti) element

Pollen spectra of Tjéri also showed small percentages (average 3.1%) of very characteristic taxa originating from more northern regions (*Artemisia* sp., *Pentzia monodiana*, *Erica arborea*, *Ephedra* sp., *Plantago* sp., *Silene* sp.). When many pollen grains are counted on successive samples of the same section, the variation of very low percentages can be used with some confidence—as, for instance, in a pollen study of the west part of the Sahara¹⁵. The pollen grains assigned to the Montane element must surely have been brought to Tjéri by the prevailing wind, that is, the harmattan, blowing from about NE or N. The water transport of pollen grains across the whole actual lake Chad is very limited⁵ and probably was so for the Palaeochad, particularly for pollen grains coming from the Tibesti, because the nearest fluvial source was about 450 km from the station studied. For these pollen grains, the nearest sources at present are some 700 km to the north on the High Plateaux of the Tibesti¹⁶. Further north, other important sources of *Artemisia*,

for instance, are found only on the narrow Mediterranean zone of Libya, about 2,000 km from Tjéri. But the latter sources seem unlikely to be involved, because other pollen types present in the Mediterranean zone of Libya, such as Cupressaceae or *Quercus*—very easily carried in the atmosphere¹⁷—would have been found at Tjéri. In conclusion, the pollen grains of the Montane element originate most probably from the Tibesti.

Comparison of the early Holocene pollen curves for the Sahelian and Montane elements shows (Fig. 3) a rough synchronicity in terms of thousands of years and a good synchronicity for the main aridity maxima. This second point shows that the percentage variability of the Montane element is not governed by variations in intensity of the harmattan, as the aridity maxima coincide with maxima of eolian activity. On the other hand, thanks to the chronology established at Tjéri, it seems that the different pollen maxima of the Montane element show good correlations with the different geological and palaeoecological events radiocarbon dated in the Tibesti (Fig. 2, Table 1). These correlations also increase the reliability of the pollen curve.

The Middle Terrace

In the Tibesti, the early Holocene coincides with the end of the accumulation of the so-called 'Middle Terrace' (MT)^{18–23} (Fig. 2). A radiocarbon dating in the lower part of the MT gave a date of 14055 ± 135 BP (ref. 20). It is possible that the base of the MT in the Tibesti began about 17500 BP like the

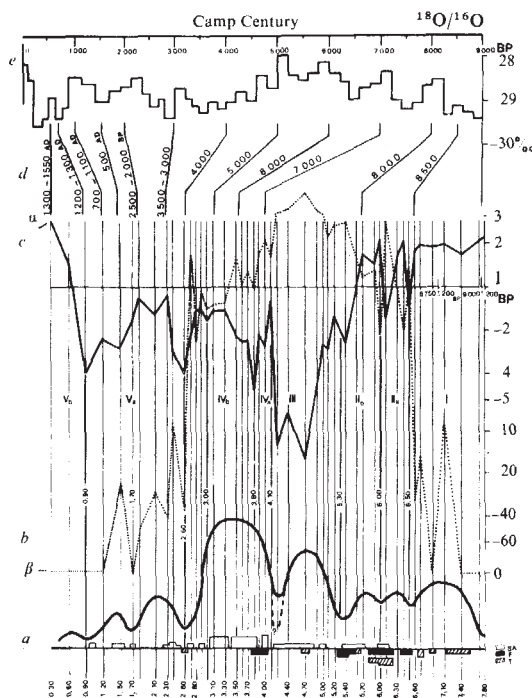


Fig. 2 Comparative evolution for the Tjéri station, from base to top, a, Relative lacustrine levels, after diatom studies^{2,14}. The ? at 4.20 m is the author's interpretation¹ and a brief regression at 3.80 m (around 6600 BP) is also probable. Some diatoms of ecological importance: SA, *Stephanodiscus astrea* var. *minutula*; F, *Cymatopleura elliptica*; T, *Cyclotella ocellata* (relative scale). b, Dotted lines, pollen curve for the Sudano-Guinean element (relative %, mean: 20.2%). β, Present time % (two samples). c, Solid line, pollen curve for the Sahelian element (relative %, mean: 33.9%). α, Present time % (two samples). These two curves (b, c) were constructed using the ratio with the mean (value of 1) carried out on the whole of the levels studied (log scales). On this scale, the value of zero was arbitrarily fixed at -100. d, Chronology reconstituted after various regional correlations. Near the base, two radiocarbon dates: at 7.75 m, 9000 ± 200 BP; at 7 m, 8750 ± 200 BP. The dates are given in BP except for the last two millennia in AD (calendar years from ref. 12). e, Schematic curve of the ¹⁸O/¹⁶O ratio for the ice core at Camp Century (Greenland) adapted from M. Ters (ref. 10); from 9000 to 1700 BP after ref. 7 and from 1700 BP to the present after ref. 6. Figure reproduced from ref. 1 with kind permission.

base of the equivalent formation in Nubia (Malki Member of Ineiba Formation)^{24,25}. In general, throughout all the Tibesti, the stratigraphic and sedimentological evolution of the MT, especially the upper part, is constant¹⁸. Some sections with radiocarbon dates facilitate the precise dating of the occurrence of the principal events intervening during the accumulation of the upper part of the MT. For the phases A, B, C, in some craters or depressions throughout the massif, there are lacustrine deposits chronologically equivalent^{18,19,26}. The accumulation of the MT occurred in several phases separated by calcareous crusts, the most recent of which were principally formed about 9200–9300 and 7600–7300 BP (D phase)^{20–22}. By correlation with the Montane element curve, it seems that the last calcareous crust formation corresponds to an arid period (see below). At the end of the D phase, a small erosion occurred before sedimentation started again. This discontinuity is a very characteristic feature. The last phase of bedded deposits (E), sometimes with coarse material, exhibits in some places, before the beginning of phase F, a thin brown palaeosol rich in organic matter^{23,26}, which was dated 6600 ± 140 BP at Mouskorbé (Gif n°3228). In Nubia, a palaeosol with kaolinite (Omda soil) was described and situated approximately at 7000 BP (ref. 24).

At the top of the MT, phase F represents a very clear sedimentological change, that is, a dramatic increasing amount of pebbles and cobbles. This phenomenon, which had started after 6600 BP, is general across the Tibesti and the Central Sahara at the top of formations equivalent to the MT. This sudden influx of pebbles is surely related to a change in climatic regime. Then the erosion of the MT (phase G) to below the actual level of the rivers could occur between about 5500 and 4000 BP. In the Tibesti, during the following H phase approximately dated 4000–2000 BP, a Lower Gravel Terrace was deposited^{18,19}.

The upper part of the MT with its main characteristics or some equivalent lacustrine deposits, can be found in many regions of the Central Sahara—for the MT, in the Hoggar^{18,27}, in Central Air³ and in Egyptian Nubia (Singari Member, upper part of Ineiba Formation and Shaturma Formation, Member I)²⁴ (Table 1). For the lacustrine deposits, the depression of Nabta Playa²⁸ (altitude about 300 m) about 100 km west of Abu Simbel, exhibits a stratigraphic succession and lacustrine phases with prehistoric occupation sites closely correlated with the events of the Tibesti (Table 1). The Terminal Palaeolithic II (or Epipalaeolithic), though without radiocarbon date, corresponds most probably to the climatic optimum B of the Tibesti (about 8400–8250 BP). On the other hand, near Bilma in the Ténéré (18°40'N–13°E; altitude about 360 m) the early Holocene lacustrine deposits³ can be correlated with phases A, B, C of the Tibesti (Fig. 3). In Adrar Bous (20°18'N–9°E; altitude about 700 m) early Holocene lacustrine deposits are radio-

carbon dated before 7310 ± 120 BP (refs 29, 30). In the middle of Serir Tibesti (23°30'N–17°20'E; altitude about 500 m) lacustrine deposits about 10 m thick are dated of the early Holocene³¹. Thus all these correlations suggest that during the early Holocene the climatic conditions were similar and apparently synchronous throughout all the Central Sahara. Therefore, the local factors seem to have had but a limited action.

One typical section of the MT for the early Holocene was chosen in order to show the correlations with the evolution of the Montane element of Tjéri (Fig. 3). This section was taken by J. Grunert²³ near Yebbi-Bou (20°53'N–18°4'E; altitude about 1,440 m) on the north side of the Tibesti. A radiocarbon-dating on shells at about 2.5 m (8180 ± 70 BP) locates this section in the chronological framework of the Tibesti and the Central Sahara.

Palaeoclimatic interpretation

To try to understand the palaeoclimates of the Tibesti in the early Holocene we can compare the pollen curves for the Montane and Sahelian elements (Fig. 3c, d). The system of representation (the ratio with the mean) facilitates direct comparison for the two curves (see other examples in refs 5, 17). Thus, from one sample to the next we can see completely opposite trends, or similar trends but of very different amplitude. Although in the scale of thousands of years there is a rough synchronicity between the curves for these two elements, the opposite trends shown up by detailed analysis imply that the rains were of different origins in the Sahel zone and the Tibesti.

For palaeoclimatological interpretations it is necessary to use as climatic models meteorological situations and climatic patterns of the present time. For instance, in Aegean regions, the climatic pattern of one winter was used to explain that of the period around 1200 BC (ref. 32); or seasonal change through the year was used to explain some aspects of the past climatic changes³³. Here the present evolution of the Saharian climate through the year is used in an attempt to establish the climatic pattern of this region in the early Holocene.

Climatic model for the present time

At present nearly all the rains falling in the Sahel zone are monsoonal in origin. In summer (July–August) these rains reach the Hoggar and Tibesti mountains^{34–36}. But, over the Central Sahara the heaviest rains fall chiefly during the intermediate seasons of spring (March–June) and autumn (September–December)^{35,37,38}. The study of the cloud formations over the Sahara also confirms the importance of the intermediate seasons³⁹. The rains of inter-

Table 1 Early Holocene events and correlations between Tibesti, Nabta Playa and Egyptian Nubia

Phases	Dates (approximate, in BP)	Tibesti	Nabta Playa (30°40'E–22°25'N) (from ref. 28)	Egyptian Nubia (from ref. 24)
H	4000–2000	Lower Gravel Terrace		
G	5500–4000	Erosion	Erosion (undated)	Erosion
F	6400–5500	Sahelian optimum		Shaturma Formation (Member I)
	~6400	Change of Climatic Régime		
E	7200–6500	Optimum: Palaeosol	Neolithic (II) 6450–7150 BP (6 dates)	Palaeosol (Omda Soil)
D	7700–7200	Erosion	Erosion	Erosion; (?) locally eolian activity, (Seiyala)
C	8100–7700	Aridity	Neolithic (I) 7850–8150 BP (10 dates)	
B	8250–8100	Optimum	Erosion	{ Fluvatile Terrace, Singari Member (upper part Ineiba Formation) 10200–8000 BP
	8400–8250	Aridity	(?) Terminal Palaeolithic (II)	
	8550–8400	Optimum	Erosion	
A	9000–8550	Aridity	Terminal Palaeolithic (I) (partly with A; 2 dates: 8580 ± 80 and 9360 ± 70 BP)	
	(?) 9300–9000			

Note that in other places the Neolithic begins in 8065 ± 100 (Tibesti), 8072 ± 100 (Acacus), 8100 ± 130 (Tassili), 8100 ± 130 BP (Hoggar)⁵³.

seasons are linked with the tropical depressions^{34,40-43} also called Sudano-Saharan depressions³⁵ or 'Khamisin' depressions in Eastern Sahara^{44,45}. Rains of this type are often fine and continuous⁴⁶, whereas the monsoon rains are stormy. At present these depressions occur rarely in winter except in Western Sahara (Senegal, Mauritania: 'Heug' rains^{34,35}). On the other hand, these depressions are absent in the height of summer when the ITCZ reaches the Sahara. Schematically the synoptic situations are as follows⁴¹⁻⁴⁶: (1) influx of polar air in the middle or upper troposphere above the Sahara along shallow troughs in the upper westerlies and (2), frequently, ahead of these cold troughs, undulations occur in the ITCZ with brief invasions of humid equatorial air. The undulations of the ITCZ could be due to the action of boreal cold troughs or to surges of monsoon caused by perturbations travelling in the Southern Hemisphere³⁶. The depressions created by the cold air aloft favour the advection of the equatorial humid air. In this part of the year the movements of these depressions are north-eastwards or eastwards³⁵. The advection of humid equatorial

an example, two Gramineae, with close taxonomic affinities, *Aristida meccana* and *A. mutabilis*, have geographic distributions which exhibit the same pattern. The first one has an area restricted to the Central Sahara, the second a Sahelian area with some stations in the Central Sahara in zones exposed to the monsoon⁴⁹. I consider that this opposition Sahel-Central Sahara, can only be explained by the hypothesis of different origins for the rains, that is, the direct monsoon and the tropical depressions. Thus this opposition must no doubt be comparable with that which existed over longer periods in the early Holocene.

Application of the present model in the early Holocene

The dominance of tropical depressions over the Tibesti seems to be proved, especially between around 8000 and 6500 BP, by the fact that the fluctuations of the curve for the Montane element approximately follow those of the present tropical depressions over the Sahara through the period of 1 yr (refs

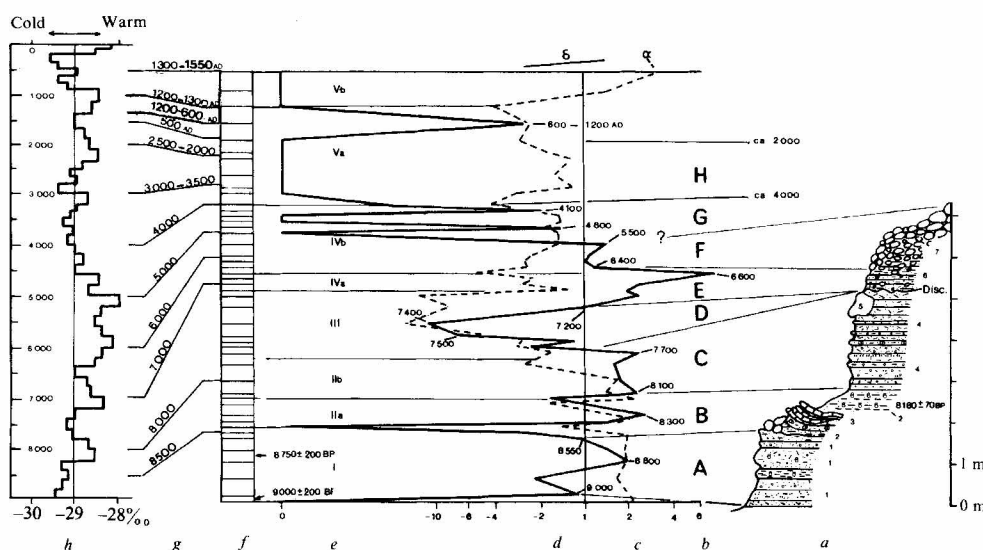


Fig. 3 Comparative evolution of the MT in the Tibesti, of the pollen curves for the Montane and Sahelian elements and of the $^{18}\text{O}/^{16}\text{O}$ ratio at Camp Century. *a*, 'Middle Terrace' (MT), section near Yebbi-Bou, after J. Grunert (23, pr. 20). 1, Indurated layers with coarse sands, bedded towards the base and becoming silty towards the top. 2, Compact silts with shells. Radiocarbon date of 8180 ± 70 BP near 2.50 m. 3, Compact indurated silt layer. 4, Alternating layers of indurated coarse sands and looser silts. 5, Boulder of calcareous tufa. Discontinuity. 6, Finely bedded layers of sand, gravels and some pebbles. 7, Conglomerate of pebbles and cobbles. *b*, Subdivisions of the MT and of the pollen curve for the Montane element of the Tibesti. *c*, Solid line, Tjéri section, pollen curve for the Montane element (relative %, mean: 3.1%). *d*, Present time % at Tjéri (two samples). *e*, Dotted line, Tjéri section, pollen curve for the Sahelian element (relative %, mean: 33.9%). *f*, Positions of the samples and two radiocarbon-dates near the base (see Fig. 2). *g*, Chronological correlations between the $^{18}\text{O}/^{16}\text{O}$ curve and Tjéri samples (see Fig. 2, *d*). *h*, See Fig. 2e.

air is essential for the formation of rain from these depressions. At present in winter, the scarcity of these depressions over the Sahara, except the western part with 'Heug' rains, can be explained by the fact that at this time of the year the ITCZ is situated at very low latitudes. But when there is interaction between cold troughs and the ITCZ, the trajectory of depressions remains chiefly over the Sudan and Sahel zones³⁴⁻³⁶.

A study of all rainfall data available since the beginning of the century for Africa north of the equator was carried out⁴⁷. It showed, by the method of spatial correlation of annual rainfalls, that in some years there is a clear opposition between the Sahel and Central Saharian zones. Either the Central Sahara gets heavy rains and the Sahel low or average rainfall, or vice versa. The phenomenon is less clear for Eastern Sahara, due to the lack of sufficient data and to the probability that the trend for the whole Central Sahara is only apparent for longer periods. Thus, for instance, only anomalies over 30 yr have a similar trend through the Mediterranean area⁴⁸. Nevertheless, this climatic opposition is genuine because it had an effect on the distribution, and perhaps the evolution, of some taxa. To give

37, 38, 45), when in the Northern Hemisphere temperatures are rising (spring) or falling (autumn). When temperatures are at their lowest (winter) these depressions are infrequent and when at their highest (summer) they do not occur. Between 8000 and 6600 BP the following correlations appear between the trends of temperatures on the Northern Hemisphere ($^{18}\text{O}/^{16}\text{O}$ curve at Camp Century^{6,7}, see above) and those of the curve for the Montane element (Fig. 3 *h*, *c*).

At around 8000–7700 BP—temperature is falling (autumn climatic model)—the Montane curve is at a maximum. At 7500–7400 BP—temperature and Montane curve are at a minimum (winter climatic model). At 7400–7100 BP—temperature is rising (spring climatic model)—there is a strong positive trend in the Montane curve. At 7100–7000 BP—temperature reaches a maximum (summer climatic model)—there is a slight drop in the Montane curve. At the same time a brief optimum occurs for the Sahelian element¹. At 7000–6600 BP—temperature is falling (autumn climatic model)—there is a strong positive trend in the Montane element curve.

Possibly this type of correlation existed before 8000 BP, but

the data available are not sufficient to allow us to demonstrate it. A consequence of this climatic pattern is that the climatic optima in the Tibesti lasted longer than those in the Sahelian zone, as is clearly evident between 8000 and 7000 BP (Fig. 3c,d). For the optima of the Tibesti, the climate consisted probably of rains fairly well distributed throughout the year, with two principal rainy seasons, one in spring, the other in autumn and with a strong reduction of the evaporation (see the lacustrine deposits). It should also be noted that the disappearance of the 'cold' diatom *Cymatopleura elliptica* in the Palaeochad (2, 14), dated after about 6600 BP at Tjéri (fig. 2) occurs just before the appearance of a warmer climate of Sahelian type (see below). Moreover during some years or periods with large seasonal contrasts (very cold winters and very hot summers), there could have been a combination of rains from tropical depressions and from the monsoon, as can sometimes be observed in present years^{37,42}. This could account for the alternation of finer and coarser layers in phase C of the MT. Finally for the climate of the Tibesti during the arid phases, of which phase D is a good example, we could perhaps imagine tropical depressions in winter and, possibly, with snow³⁵, the rest of the year being almost without rain, which would cause intense evaporation favouring the formation of calcareous crusts. Cyclonic rains from the Polar front in surface are also possible in winter without the intervention of equatorial air. But since the cold air carries little humidity, these rains would in general be relatively light^{34,50}.

Interpretation of the upper part of the MT

From around 6500 to 5500 BP, the relationship with temperature seems to be different (phase F). The trends of the curves for the Montane and Sahelian elements become similar. One may thus suppose that during this time the Tibesti underwent the same regime as the Sahelian zone, which means that almost all rains would be provided by the summer monsoon.

First, the sudden massive appearance of the pebbles and cobbles near the top of the MT is subsequent to 6600 BP, as the palaeosol radiocarbon dated about 6600 BP is itself covered by 40 cm of coarse alluvium²⁶. On the other hand, the spreading of the pebbles requires a very heavy fluvial regime for which rains of Sahelian type can easily be accountable. However one must note that this spreading occurred over the MT without apparent erosion. The erosion period of the MT is not yet well placed in time: perhaps after 5500 BP and before 4000 BP (Fig. 3). In Central Air, the presence of vertisols with calcareous nodules of dates 5680 ± 110 and 5010 ± 110 BP (ref. 3) indicate a wetter climate of Sahelian type. In Adrar Bous a vertisol has been situated between 7300 and 4500 BP (ref. 30). In Egyptian Nubia Member I of the Shaturma Formation²¹ has a chronology and sedimentology corresponding to the phase F of the Tibesti. The alluviation of Member I ceased before the initiation of a renewed period of Nile dissection which occurred after 5000 BP (ref. 24). The end of Member I and the beginning of the erosion can thus be dated between around 5500 and 5000 BP. Butzer and Hansen²⁴ conclude that these deposits "imply rather effective sheet-flooding with 50 to 100 mm of rainfall at most".

A climate of Sahelian type over the Tibesti and probably also over the Central Sahara is all the more probable as other pollen data at Tjéri indicate a northwards movement of the Sahel zone at that time. The removal of the Sahelian vegetation northwards would explain the flat of the curve for the Sahelian element between around 5500 and 4400 BP (Fig. 2c). This interpretation is corroborated by the curve for the Sudanian element which reached its maximum between around 5900 and 4400 BP (ref. 1), corresponding no doubt also to a slight northwards movement of the Sudanian zone.

Conclusion

First, it seems that for the Central Sahara there are two kinds of wetter periods (optima)—one, more frequent in the Quaternary period, with tropical depressions and temperature relatively

low, and the other with direct monsoon rains and higher temperatures. Second, the annual climatic mechanisms of the present time enable the palaeoclimates of the early Holocene to be coherently explained. The opposition, both seasonal in the present time, and for longer periods during the early Holocene, between the tropical depressions and the monsoon rains can be interpreted by variations in trends of temperature over the Northern Hemisphere and also by variations in general atmospheric circulation. Indeed, the penetrations of the polar troughs aloft, necessary for the formation of tropical depressions, are related to the occurrence of large amplitude waves in the upper westerlies. On the other hand, during hotter years or periods, the polar troughs towards lower latitudes are much less frequent and the circulation of the upper westerlies becomes more zonal. This situation leads to a diminution of tropical depressions, favouring, in contrast, the extension of monsoon rains over the Sahara^{51,52}. But the opposition between these two kinds of rain remains only partial since both are also linked to the activity of the ITCZ. Better knowledge of present events as well as the study of other old periods should allow greater precision in this field.

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Dispersal in stable habitats

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Simple mathematical models show that adaptations for achieving dispersal retain great importance even in uniform and predictable environments. A parent organism is expected to try to enter a high fraction of its propagules into competition for sites away from its own immediate locality even when mortality to such dispersing propagules is extremely high. The models incidentally provide a case where the evolutionarily stable dispersal strategy for individuals is suboptimal for the population as a whole.

IN nature, adaptations for dispersal are ubiquitous and are often applied in almost suicidal ventures or in ventures which seem too feeble to be worthwhile (such as dehiscent seeds that fall only a few feet from the parent plant). This behaviour is clearly advantageous if the habitat is unstable or offers many empty, if transient, patches. We discuss here some simple models that help to explain why such dispersal is also advantageous even in stable and saturated habitats.

To begin, we consider a wholly parthenogenetic species in an environment that provides a fixed number of sites, at each of which just one adult can live. In a fixed season at the end of its life each adult produces a certain constant number of offspring, m . A fraction v of these are programmed to be migrants (for example, insects provided with wings and appropriate instincts, seeds with a pappus for wind dispersal). The remaining fraction $(1-v)$ are destined to be sedentary competitors for the home site. The mother's genotype, by means of some maternal influence on each ovum (or testa or fruit), determines the fraction v . When ready, at about the time of the mother's death, the migrant offspring take off and after mixing with all other migrants, and suffering a mortality such that only a fraction p survive, they are distributed equally to all the sites. There they compete on equal terms with the resident young which, it is supposed, up to this stage suffer no mortality (alternatively, if they do, p is the relative survival of the migrants). In effect, one offspring is chosen at random from among the young present on a site to become the adult at that site in the new generation.

We acknowledge that this simple model probably has few close parallels in the real world. Nevertheless it may usefully force a re-examination of some widely held ideas about migration.

For example, it has been claimed as "intuitively obvious" that in a saturated and time-invariant environment "organisms can never gain any advantage by changing their locations"¹. The schematic illustration presents a counter-example based on the above model

				x		x		
	o	o	o		o	o	o	o
Offspring	o	o	o		o	o	o	o
(after dispersion)	o	o	o		o	o	o	o
	o	o	o		o	o	o	o
	o	o	o	x	o	o	o	o
Adults	O	O	O	X	O	O	O	O
Site labels	a	b	c	d	e	f	g	h

The environment (represented by the eight sites labelled a to h) here is saturated and time-invariant. In the absence of any migrating mutant like X , and with full survival of stay-at-home propagules, the majority genotype O which keeps all propagules at its own site can perfectly maintain the population. But the 'O' strategy is not evolutionarily stable. The genotype O will be replaced by the mutant X , which keeps only one propagule at home, even though it loses two of its remaining four propagules due to mortality in migration (assumed to be 50%; $p = 0.5$): obviously X has a chance of $1/6$ of winning each of sites e and f ; meanwhile, at least against so ill-advised a genotype as O , it certainly retains its base at d . Hence X is certain to become the established type. Of course the particular migration probability of X ($v = 4/5$) illustrated here will not itself prove to be the evolutionary stable strategy, or "ess"², except for some special value of the survival factor p . Normally other mutations would supervene, after the spread of X , until finally a migration probability that was evolutionarily stable was approximated.

In general, the one or more ess migration probabilities which the model might have can be determined as follows. The population is imagined to contain two types, using migration fractions v and v' . An expression is written for the fitness, w' , of one adult of type v' . This fitness, or expected number of sites to be gained by offspring, will consist of the chance of retaining the home site plus the expectation of sites to be gained elsewhere by migrant offspring. From this we find the value of v which has the property that $w' \geq 1$ for all $v' \neq v$ (mean fitness in the model is unity, so that $w = 1$). The value of v so found, symbolised v^* , is unbeatable³ in the sense that any genotype with strategy $v' \neq v$ will have a diminishing frequency in any mixture.

For the simple limiting case where the number of sites and the number of propagules per parent are large, we find by this method that the ess or unbeatable migration probability is

$$v^* = 1/(2-p) \quad (1)$$

Because this formula shows no dependence on the composition of the mixture, stable mixtures are not possible, and the ess can be considered safe against both rare mutations and any massive invasion by a different genotype.

A striking conclusion to be drawn from equation (1) is that even when migrant mortality is extremely high (small p), and the environment offers no vacant sites for colonisation, it is still advantageous to commit slightly more than half of the offspring to migration.

Pre-eminent among the artificialities of this model are: (1) insistence on death and replacement of every parent in each generation; (2) absence of vacant sites (stemming from the deterministic description of the propagation processes); (3) pure parthenogenesis. We now indicate how the model may be extended to encompass such effects, paying particular attention to (2).

Death and replacement of parents

There is one simple and often realistic assumption that allows for perennation of the parents, while preserving the result (1). The assumption is that all parents, irrespective of age, have

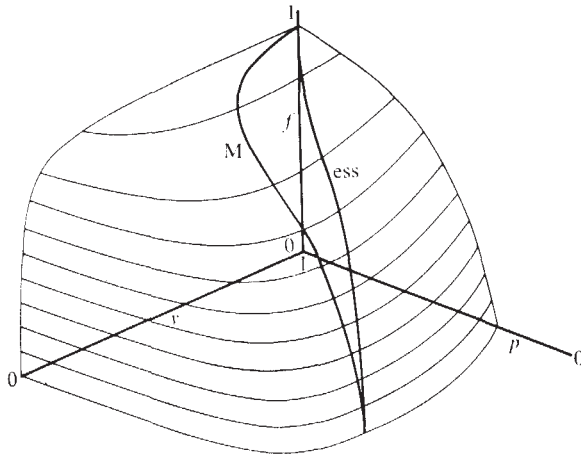


Fig. 1 The surface depicted here gives f (the equilibrium fraction of sites occupied) as a function of v (the fraction of offspring that migrate) and p (the relative survival probability for migrants), under the assumptions delineated in the text, for a mean 'brood size' $m = 3$. The contour lines are for constant values of f , spaced at intervals 0.1 apart (that is $\Delta f = 0.1$). On this surface, the trajectory labelled *ess* shows the dispersal strategy that is evolutionarily stable, $v^*(p)$, as a function of p . The *ess* trajectory is to be contrasted with that labelled *M*, which shows the strategy which maximises site occupancy for given p , $\hat{v}(p)$.

some constant probability q to survive and retain their sites into the next season. This corresponds to the so-called 'Type II survivorship curve', which is known to be not far from true for many organisms. For such organisms, both stay-at-home and migrant offspring have their expectations of inheriting a vacant site devalued by exactly the same factor, namely $1 - q$, which consequently cancels itself out of the analysis. (On the other hand, for organisms whose chance of surviving from one year to the next diminishes with age, the *ess* will be age-dependent, if this is biologically feasible. A likely outcome is a 'bang-bang' strategy⁴, with all propagules dispersed until their parent reaches a certain age, whereupon all propagules stay at home.)

Vacant sites

In our simple model, the evolutionary pressure that commits at least half the offspring to migration, no matter how risky migration is, results from an advantage in arranging competitive interactions as far as possible with unlike genotypes. It can be imagined that in nature such a pressure to take risks could cause a wastage that was damaging to the population's chance of survival. This cannot happen in the simple model because its deterministic assumptions keep all sites occupied; to investigate wastage we need some version that relaxes this assumption (2). Such a version may have some bearing on our confidence in models of theoretical ecology in general, because these often take it for granted that the species discussed already have, or will evolve towards, maximal efficiency of resource utilisation.

To this end, we stochasticise the earlier model by assuming the number of propagules produced at any given site is given by a Poisson distribution, with mean equal to the previously constant value m . It follows that the numbers for emigrant and for stay-at-home offspring, and for immigrants, are all generated by Poisson distributions for which the same symbols and expressions as occurred in the simple model are now to be taken as the means. Note that m is the mean brood size and also the variance in brood size, so that the relative magnitude of statistical fluctuations goes as $m^{-1/2}$. The parameter m thus comes to have an important influence, especially when relatively small; when m is large, the present stochastic model tends to revert to the earlier deterministic one.

Now sites can become vacant. Vacancies begin or continue

whenever a site happens to have no surviving stay-at-home offspring and receives no immigrants. Obviously, some migration is essential if extinction is to be avoided, and from the population point of view there will be some level of migration that keeps extinction at furthest reach⁵.

For any specified combination of m , v and p , the system settles to some stable level of site occupancy f (that is f = fraction of sites occupied), with a zero level signifying that extinction is inevitable. This stable level f is given (for $f > 0$) by the implicit relation

$$f/(1-f) = e^{m(1-v)} [e^{mvpf} - 1] \quad (2)$$

For given m , the site occupancy function f defined by equation (2) may be mapped over the unit square of admissible values of v and p : $f(v, p)$ is found to have the form of a smooth promontory (Fig. 1 for $m = 3$), or, for larger m , a squarish headland (Fig. 2 for $m = 10$). In any such figure, for fixed v the occupancy $f(p)$ falls convexly with decreasing p , and clearly must always hit extinction somewhere short of $p = 0$. The smallest p that still allows the population to exist may be shown to occur when $v = 1/m$, that is when just one migrant is dispatched from each site. The concomitant least value of p is given by $p_{\min} = \exp(1-m)$, which is very small for moderate values of m ; slightly larger values of p carry f up to a 'plateau' at a level of nearly complete occupancy. Conversely, for fixed p , the occupancy f as a function of v at first rises (albeit very slightly for large m) to a maximum as v decreases; then, as v decreases further, this is followed by a steepening fall in f , which goes to zero short of $v = 0$.

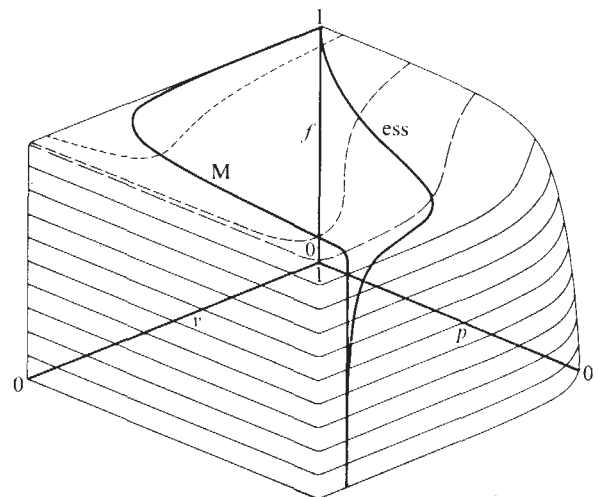
Thus we may trace out the locus of the strategy, $\hat{v}$, that maximises the site occupancy f for given p ; this locus moves up a 'cliff', and across a 'plateau' to $p = v = 1$ (Figs 1 and 2). This strategy $\hat{v}$ is that which is 'best for the population'.

This locus of the strategy for maximal occupancy, $\hat{v}$, is to be contrasted with the locus of the *ess* v^* , which is determined by the method described above. The *ess* migration probability, $v = v^*$, is given by

$$\exp[m(u + vpf)] = \frac{v^2p - vpf(u + vpf)}{v^2p + u(u + vpf)(1 - mv)} \quad (3)$$

Here $u = 1 - v$, and f has the value implicitly fixed by equation (2). The derivation of the results (2) and (3) will be set out

Fig. 2 As for Fig. 1, except that now the mean brood size is $m = 10$. Again the solid contour lines depict constant values of f , from $f = 0$ to 0.9 at intervals of 0.1; the increasingly broken contour lines on the 'plateau' are for $f = 0.99, 0.999$ and 0.9999 , respectively. For a more full discussion of Figs 1 and 2, see text.



elsewhere (our work with H. N. Comins); unlike the earlier simple result (1), these equations are made more complicated if age-independent or 'Type II' perennation of the parents is introduced.

The locus of the ess migration fraction v^* , so determined, is shown in Figs 1 and 2. We note that ess migration is always higher than maximum-occupancy migration, although they converge at the two extremes (at perfect and at least-possible survival, $p = 1$ and $p = p_{\min}$). This is true for all brood sizes m . As m is increased towards more usual biological values (from $m = 3$ in Fig. 1 to $m = 10$ in Fig. 2), the separation between the ess and maximum-occupancy loci with respect to v also increases strikingly. This separation results mainly from the way the changing shape of the site-occupancy surface $f(v, p)$ controls the course of $\hat{v}$; that is $\hat{v}(p)$ is much more affected by changing m than is $v^*(p)$. The separation between the two loci with respect to f , however, remains very small, even for populations that are on the 'cliff face' of the f surface and hence in some danger of extinction.

In other words, the ess v^* can demand far more migration than is 'best for the population' ($\hat{v}$), but such excess does not, in this model at least, substantially affect population safety nor create many extra vacant sites that would give other species opportunity to evolve as competitors for the niche. Notwithstanding the small difference in occupancy levels, there is usually strong selection to establish v^* rather than $\hat{v}$. One incidental consequence of the relatively abundant supply of migrants is that some must overflow from the system and occasionally have the luck to found new populations elsewhere. It therefore seems that our models show, after all, a case where a trait is positively adaptive at more than one level: the ess is best for the genotype, suboptimal at the level of an isolated population, and advantageous in carrying the population to new areas.

Sexual organisms

If the organisms are taken to be sexual rather than purely parthenogenetic, with migration probability depending on the genotype of the offspring, lower values of v^* are definitely expected.

This follows from the observation that our models directly treat the genetic success of a mother. A parthenogenetic offspring has an identical genotype, so that whatever maximises inclusive fitness⁶ for the mother maximises it for the offspring. In contrast, the inclusive fitness of a sexual offspring normally differs from that of its mother, and, in some direct relation to the hazards of migration, will find its optimum at a lower migration rate. Hence there is a conflict of interest between parent and offspring^{7,8}. Its outcome will depend on the biological circumstances: as regards applying a pappus to a seed to make it blow away it is clear that the parent has a strong position; as regards making wing buds become wings in an insect it is much more likely that the offspring can have its preference.

In nature, less migration generally means more inbreeding; such inbreeding reduces the genetic contrast between parent and offspring, and thus tends to diminish the difference between parthenogenetic models and sexual models with offspring-determined migration. Various situations can arise. If males mate before migration in a one-per-site situation, inbreeding is total and the model is again effectively as under parthenogenesis. Unfortunately, the small arthropods which almost meet this extreme of inbreeding and which have a dispersal polymorphism (for example, some mites of the genus *Pygmephorus* (ref. 9 and unpublished results of W.D.H.)) are far from meeting the assumption of fixed unitary sites, being rather exploiters of patchy, ephemeral resources. If inbreeding occurs because males sometimes survive along with a sister, then our one-per-site assumption does not hold.

But if male offspring are assumed always to migrate, then outbred sex can be brought in and the one-per-site feature

retained; moreover, this version may approach realism for some of the many insect species that have free-flying males while females are either wholly flightless or polymorphic for flight. For the simple, non-stochastic version of this model with allele semi-dominance, the ess migration probability for the female offspring is indeed lower than for the parthenogenetic model: in place of equation (1), we now have

$$\begin{aligned} v^* &= 0 & \text{for } \frac{1}{2} > p > 0 \\ v^* &= (2p-1)/(4p-1-2p^2) & \text{for } 1 > p > \frac{1}{2} \end{aligned} \quad (4)$$

The fact that $v^* = 0$ for $p < \frac{1}{2}$ suggests that when this model is made stochastic (along the lines followed previously) the ess may imply situations where the population and the species is dangerously liable to extinction. We are pursuing this open question.

(Roff¹⁰ has used computer simulations to study migration in models which not only have sexual reproduction and multiple adults per site with a density-dependent migration structure, but also have temporal variability in site suitability. It is obvious from the facts of nature¹¹, and is confirmed in both Roff's model and several others^{1,12-14}, that erratic habitat suitability (including local extinction) is an extremely important factor in favouring adaptations for dispersal and migration. More relevant to the present discussion, however, is Roff's demonstration that his ess migration probabilities are considerably lower than those which would keep populations highest and most secure from extinction. Although partly attributable to the workings of sexuality and Mendelian inheritance in Roff's models, this effect arises primarily because there are many adults per site, which changes the calculation of inclusive fitness in such a way as to favour the short term, selfish option of not dispersing.)

It would be satisfying to conclude by listing a few biological illustrations of principles suggested by the models. Unfortunately most of the examples which have come to mind are more in the region of jokes than of reality: they range from *Caulobacter crescentus*¹⁵, whose asexual fission always produces one stalked sessile cell and one motile one that swims away, through the inevitable lemmings and on to the non-first sons in Victorian families who joined the army or went to Australia. Both our simplest model, and the refined versions (1) and (2), support what these examples might illustrate, namely a minimum rule that 'at least one must migrate whatever the odds'. All three examples, however, fail to have fixed sites and, except for *Caulobacter*, fail also in regard to asexuality. There are flight dimorphisms in non-sexual insects (for example, aphids in summer: the tiny sub-cortical beetle *Pinella errabunda*¹⁶) and probably parallels in some weeds (for example, Compositae)^{17,18}, but in being colonist species these fit badly with the assumptions of fixed sites and of no extinction or recreation of habitats on a large scale. But the fact that colonist plants in arid environments, where perennation of the whole plant (as opposed to survival of its seed) may be hardest to achieve, seem especially inclined to produce actually dimorphic or differently shed propagules with one class buried or otherwise retained beside or under the parent¹⁹ (*Sieglingia decumbens* is the best example in Britain) does at least suggest that a plant's perennating body (or its tubers, corms or the like) should be considered its bid to retain the home site. We expect the investment in such aids to perennation, as opposed to investment in flowers and dispersing seed, to show positive correlation with the chance that seed if produced would end up destroyed or in sites that never could support an adult. For example, the more successful a specialised epiphyte or parasite is at getting its propagules to suitable sites, even when these are objects of intense competition, the more it should produce them. A mistletoe should flower more than an epiphytic orchid; and we should not be surprised to find that weedy perennial species put more net assimilation into seed production than do climax perennial species²⁰. This may be so but is not particularly exciting; other explanations are available. It is not easy to test the quantitative conclusion that,

because $v^* > \frac{1}{2}$ for most values of p , we expect to find at least one-half of net assimilation going into some form of growth or propagule production that extends competition beyond the space that the organism already occupies.

The value of our models lies primarily in their approach and method, in their well-determined conclusions, and in the novelty of having made plain a new reason for the ubiquity of dispersal. We have shown that the habitat does not have to be patchy and of erratic suitability; substantial dispersal is to be expected even when the habitat is uniform, constant, and occupied completely.

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letters to nature

Radio emission from a normal HD26676 star

IN the field of an observation of the cluster Abell 478 made with the Westerbork Synthesis Radio Telescope¹ at 21 cm, we have discovered and report here a weak radio source coincident with the 6.2-mag B8Vn star² HD26676. The source positions derived from both the 21-cm observation, and measurements made later at 6 cm, agree within the errors with the AGK3 position of the star (Table 1). The 21-cm data were obtained during a single 8-h observation, while at 6 cm three measurements, each lasting for 10 h, were made over a period of 6 weeks. There is no significant indication of variability from the three flux densities derived at 6 cm (Table 2). The global 6-cm flux density, as well as the position in Table 1, were obtained by combining the three observations in a single map.

Polarisation (linear and circular) is less than 10% of the total intensity at 21 cm and less than 20% of that at 6 cm. The radio source is unresolved at both wavelengths, which sets an upper limit (in right ascension) of about 4 arcs to the angular size. No radio emission coincides with the reflection nebula associated with the star³ at a level of about 2 mJy per beam (area about 1 arc min²) at 21 cm and about 1 mJy per beam (area about 0.1 arc min²) at 6 cm.

Although the optical and radio positions agree, within the errors, we must consider what the probability of a chance coincidence is. The area enclosed by the 6-cm position errors is about 2×10^{-6} deg². The density of stars brighter than 8 mag (ref. 4) (we assume that anyone finding a radio source close to such a star would be prompted to investigate the possibility of association) is about 1 deg⁻², suggesting an *a priori* probability of 2 in 10^6 that the coincidence occurred by chance. While this is reasonably small, thorough identification work has been

done on nearly 1,000 radio sources observed with Westerbok at 21 cm (see ref. 5 and refs therein), and one other radio position agrees with that of a 13-mag star. Nevertheless, we conclude that the star and radio source are probably related and now consider the consequences of this fact.

Assuming the source did not vary during the 10 months separating our two sets of observations, the flux densities in Table 2 give a spectral index between 6 cm and 21 cm of $\alpha = -0.8 \pm 0.2$ (where flux density is proportional to frequency α). This qualifies HD26676 as a radio star according to the definition of Hjellming *et al.*⁶: if the source has not varied, its radio spectrum clearly indicates that the radiation is not the result of free-free emission from a surrounding nebula. Like several of the other radio stars (such as β Per and β Lyr), HD26676 is of an early spectral type. At a distance of 126 pc (ref. 3) its 21-cm luminosity is 2×10^{10} W Hz⁻¹. The radio power between 1 GHz and 10 GHz (assuming a constant spectral index) is 8×10^{26} erg s⁻¹, a value typical of the 'quiet' state of radio stars.

Table 2 Measured radio flux densities

Wavelength (cm)	Mean date	Flux density (mJy)
6	6.5 February 1977	5.8 ± 1.2
6	21.9 January 1977	3.3 ± 1.1
6	27.9 December 1976	4.3 ± 1.1
6	(combined)	4.1 ± 0.6
21	12.8 February 1976	11 ± 2

In several respects HD26676 differs from other radio stars. Its spectral index is negative, while that of radio stars in their quiet state is usually positive. But there is evidence that the radio flares must be non-thermal⁸ and in several cases, notably Sco X-1 (ref. 9), the spectral index is generally negative. Of course, Sco X-1 is predominant as an X-ray star, and the SAS-3 observation of Abell 478 (ref. 1) enables one to put a limit on any X-ray emission from HD26676. H. W. Schnopper (personal communication) has estimated that the count rate cannot exceed one-third of that from Abell 478. This means that the 2–11-keV luminosity of HD26676 must be less than a few times 10^{31} erg s⁻¹ (epoch 1976.9). This is considerably less than the luminosity of typical X-ray stars such as Sco X-1, Cyg X-1 and Cyg X-3, all of which are also radio sources.

Table 1 Optical and radio positions

Observation	Right ascension (1950.0) (h min s : s)		Declination (1950.0) (° ' " ± ")	
Optical*	04 10	50.11 ± 0.01	10 05	11.54 ± 0.2
6.0 cm	04 10	50.19 ± 0.11	10 05	04 ± 9
21.2 cm	04 10	50.14 ± 0.15	10 05	07 ± 16

*The position given is corrected for proper motion to 1977.0

The most important difference between HD26676 and all other radio stars is the fact that it is not known to be either a binary system or a variable star. Most models of the radio emission make use of mass exchange between stars in the system⁷. For this reason HD26676 could be a critical test case for emission mechanisms. If further observations confirm the radio-optical association and its non-binary character, it would provide an important constraint for future models.

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Enhanced metal depletions and interstellar H₂ abundances

THE OAO 3 Copernicus ultraviolet satellite has provided important new data on the abundances of elements in the interstellar gas in the direction of many O and B-stars along lines of sight of low optical depth¹⁻⁴. This information may make it possible to choose between the following theories of molecular hydrogen formation in interstellar clouds: (1) physical adsorption of H-atoms on to cold dielectric grains and their subsequent recombination and desorption⁵; (2) H₂ recombination on graphite grains⁶; and (3) hydrogen recombination by non-activated chemisorption on transition metal grains⁷. Indirect methods of testing the above theories are essential because there is a glaring lack of experimental data, especially for processes (1) and (2). Also, it does not seem possible that in the near future radio astronomers will be able to measure the abundances of the various hydrogen ions (such as H₂⁺ and H₃⁺) that the above theories predict⁸.

In view of the fact that the catalytic production of H₂ can take place in the laboratory⁹, it probably occurs in the interstellar medium because the temperatures of metal grains are about an order of magnitude hotter than dielectric ones¹⁰. An examination of the depletion of interstellar elements from the gas phase may ultimately be able to discredit or lend credence to this theory. Consider Table 1, which lists the interstellar depletions with respect to solar system abundances ($\delta = \log(N/N_H) - \log(N/N_H)_\odot$) along lines of sight to ζ Oph (ref. 2), o Per (ref. 4), and λ Sco (ref. 3) for certain key elements—carbon, oxygen, silicon, and iron. Towards ζ Oph and o Per, where the

visual extinction $A_v \approx 1.0$ mag, the fraction f of hydrogen that is in molecular form is between 0.5 and 0.7, while towards unreddened λ Sco, $f \sim 10^{-7}$. There seems to be a weak correlation between iron and silicon depletions and H₂ abundances. In contrast there seems to be no noticeable relationship between the amounts of carbon and oxygen depleted from the gas phase and the amount of gaseous H₂ detected. Since it is commonly assumed that process (1)—the physical adsorption of hydrogen—is the primary mechanism for H₂ production, one might have expected to find enhanced carbon and oxygen depletions with the higher abundances of H₂. The reason for this preconceived notion is the assumption that the cold dielectric grains where this process is thought to occur are mainly silicate core particles with heavy mantles of C-N-O 'ices'⁵.

These conclusions are partially reinforced by an examination of depletions in high-velocity clouds. Barlow and Silk¹¹ have shown that there is a systematic depletion of iron and silicon with gas velocities towards unreddened stars: the higher the velocity, the lower the amount of depletion. They make a strong case that this is the result of the sputtering of refractory grains in interstellar shocks. It is interesting to note, however, that the graph of the depletion factors for iron and silicon plotted against the logarithm of gas velocity has the same characteristic shape (but opposite slope) as a plot of $\log N_H$ (cm⁻²) against $\log v$ (km s⁻¹). This effect may be statistical in that Barlow and Silk used data from only seven stars. It is also reasonable to assume that this pattern is not a coincidence and that higher gas velocities always correspond to an increase of iron and silicon in the gas phase and a decrease of atomic hydrogen, as explained by their 'snowplow' model¹¹.

An examination of the data for these seven stars¹¹⁻¹³ shows that although there is a good correlation between the δ s and gas velocities, there is only a very weak correlation between the δ s for iron and silicon and the values of f . For example, for the low-velocity component of μ Col (ref. 12), $\delta(\text{Fe}) = -1.8 \pm 0.2$ and $f \approx 4.5 \times 10^{-5}$, while for the higher-velocity component, $f \approx 6 \times 10^{-7}$. Unfortunately, for HD74455 (ref. 13), $\delta(\text{Fe}) \sim 0.1$ while $f \sim 4 \times 10^{-5}$. Part of the problem is that towards all these unreddened stars, $10^{-4} < f < 10^{-7}$, and a small error in observation or reduction can produce a correspondingly much larger error in f . Also, these stars may have fairly recently undergone heating by interstellar shock fronts, producing large deviations from equilibrium abundances. Thus, studies attempting to correlate $\delta(\text{Fe})$ with H₂ abundances should confine themselves to low-velocity clouds of appreciable ($A_v \approx 1.0$ mag) extinction.

If we do accept the possibility that there is a direct correlation between the amount of H₂ being produced and the amount of iron and silicon in the grains, we are still left with two alternatives. First, some of the H₂ may be formed by means of catalytic reactions on the surfaces of iron grains (process (3)). Although nothing in the above analysis proves this conclusively, nothing contradicts it either. Second, H₂ may be produced by process (1) on cold, silicate grains through the mechanism of physical adsorption. Although it may be possible for reasonably pure iron grains to exist in the interstellar medium by virtue of the fact that metallic iron will condense out of a hot gas before any of its compounds, the same cannot be said for silicon. Studies of the condensation temperatures of compounds and elements¹⁴⁻¹⁶ show that pure silicon is an unlikely possibility. Instead, silicon forms such compounds as CaAl₂Si₂O₈, Ca₂SiO₄, and Mg₂SiO₄¹⁷. Thus, for every atom of silicon that is depleted, there is an average of 3-4 oxygen atoms. As oxygen is about 21 times more abundant cosmically than silicon¹⁸, there is still

Table 1 Interstellar depletions of elements

Star	E(B-V) (mag)	$\delta(\text{C})$	$\delta(\text{O})$	$\delta(\text{Si})$	$\delta(\text{Fe})$
ζ Oph	0.32	-0.87 to -0.54	-0.68 to -0.49	-1.88 to -1.38	-2.05 to -1.89
o Per	0.32	-1.65 to -0.43	-2.70 to -0.59	-2.13 to -1.82	-2.39 to -2.14
λ Sco	0.03	+0.2 to -0.9	-0.7 $\pm$ 0.1	-1.1 $\pm$ 0.1	-1.3 $\pm$ 0.2

plenty of oxygen left to be incorporated into extensive 'ice' mantles and still agree with the interstellar depletions listed in Table 1. Hence, nothing in Table 1 contradicts the possibility of process (1). (As for process (2), nothing can be said at this time since there is a strong possibility that crystalline graphite cannot be produced in the interstellar medium^{19,20}.)

Observations of interstellar depletions may one day be able to decide how H₂ is produced in interstellar clouds. At present all that can be said is that neither process (1) or (3) can be dismissed without further consideration. It is hoped that this paper will stimulate additional research.

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Interstellar grains as possible cold seeds of life

As long as entropy ceases to play any part in equilibria when the temperature approaches absolute zero, all exothermic reactions—up to the formation of the most complex biopolymers—become thermodynamically profitable. In such conditions the reaction rate starts to play a decisive part in the observability of chemical transformations. According to classical views (Arrhenius law) all reactions should completely stop when $T \rightarrow 0$ and therefore the cold synthesis of biologically active substances is absolutely excluded. The phenomenon of a low-temperature limit to chemical reaction rates found recently, however, in the studies of radiation-induced polymerisation of formaldehyde¹⁻³, showing the existence of chemical reactivity even at $T \rightarrow 0$, obviously caused by quantum-mechanical molecular tunnelling, makes it possible to combine complete suppression of the entropy term in equilibria with an observable rate of exothermic chemical reactions, for example, of chain polymerisation (or any other integration of molecules) induced by light or ionising radiation. Thus molecular tunnelling suggests the possibility of a cold pre-history of life¹⁻³.

The possibility of synthesising rather complex molecules under the combination of deep cosmic cold and various radiations of cosmic origin was recently proposed by Wickramasinghe⁴⁻⁶ and Hoyle and Wickramasinghe⁷. Basing their argument on the properties of galactic nebulae in the infrared wavelength region they concluded that formaldehyde undergoes polymerisation in interstellar space with the formation of polyoxymethylene and even of polysaccharides.

As discussed elsewhere⁸, the only possible mechanism of interstellar formation of such complex substances must be molecular tunnelling. From the results given in refs 1-8 one can assume that the grains of interstellar dust clouds serve as cold seeds of life.

Interstellar chemical reactions have attracted the interest of astronomers, physicists, chemists and biologists (see refs 9-15).

Attention has been paid mainly to the gaseous reactions in interstellar clouds as well as to the reactions at the surface of grains. Most data concerning the formation of complex n -atomic molecules for $n \leq 5-9$ were explained by the mechanisms of bimolecular ion-molecule, or radical-radical, or radical-molecule interactions.

All authors, however, agree that the generally accepted mechanisms listed above are insufficient and unsatisfactory for $n \geq 5-9$. So clearly, these mechanisms should not be applied to the formation of polyoxymethylene and more complex compounds.

It seems necessary to consider interstellar grains as possible cold seeds of life, with chemical reactions in the bulk of these grains as a very important stage of prebiotic evolution. The idea of a very significant role of reactions in 'dirty-ice' mantles of grains in the formation of quite complex molecules (up to amino acids) was put forward and defended by Greenberg (see refs 11, 13, 16). He regards as a main process leading to the integration of molecules the recombination of ultraviolet-produced radicals and other active centres assuming that such processes can lead to the explosion of grains once the over-critical concentration of active centres is reached. Besides emphasising that the action of any permanent external ultraviolet source can lead only to saturation in the yield of radicals rather than to their over-critical concentration^{8,17}, one should remember that the recombination of radicals cannot form more complex products than can the other mechanisms mentioned here. I do not discuss here the role of shock wave-induced polymerisation and polycondensation of solids (up to the transformation of amino acids in polypeptides) we have observed¹⁸⁻²⁰.

The cold pre-history of life, of prebiotic evolution in interstellar clouds postulated here is based on several main points. (1) The formation of complex molecules—up to the biopolymers—proceeds in the 'dirty-ice' mantles of interstellar grains in diffuse or dense clouds at very low ($T \sim 10-20$ K) temperatures. The decisive part in such formation is played by exothermic (particularly chain-type) processes of addition and polymerisation proceeding by molecular tunnelling.

(2) Integration of molecules in surface regions of clouds is ultraviolet-initiated. As shown elsewhere⁸, the appearance of long chains ($v \geq 1$) of chemical transformations is possible in such conditions only if the duration of an elementary act of continuation of chain $\tau_E \ll R_{act} \tau_{uv}$, where $R_{act} \sim 10^{-3}$ to 10^{-2} is the saturated concentration of active centres, that is, the time spent by each molecule in an active state under the external ultraviolet-irradiation. $\tau_{uv} \sim 100$ yr is the characteristic time between two successive ultraviolet absorptions by any molecule of the grain. Thus for the development of long chains $\tau_E \ll 0.1-1$ yr.

(3) Integration of molecules in the depths of clouds can be initiated only by long-range cosmic protons. As the characteristic time between two successive interactions of such protons with any molecule of the grain $\tau_p \sim 3 \times 10^9$ yr is 10^3-10^4 times longer than the life-time of dust clouds τ_{cl} limited by their gravitational collapse or by cloud-cloud collisions (10^6-10^7 yr)^{4,14} the accumulation of active centres in the depths of clouds does not reach saturation. Therefore, the development of chains of reactions requires the much less stringent condition: $\tau_E < (1/v) \tau_{cl}$ and one can expect the formation of more complex molecules, and the occurrence of much more advanced prebiotic evolution in the depths of clouds rather than at their surfaces and in the interstellar gas.

(4) The equilibrium chemical composition of cold interstellar dust can be obtained by relatively simple calculations under the assumption that entropy factors can be neglected and that the whole totality of chemical reactions leads to the formation of the system of monomers with a minimum possible enthalpy (for the given balance of abundances of various elements).

The possibility of existence of some unsaturated H, C, N, O (and also S, P)-containing monomers (with multiple inter-

atomic bonds) in such equilibrium dust would automatically mean also the possibility of exothermic polymerisation of such monomers (like the case of polymerisation of formic acid and methanimine with formation of glycine mentioned in ref. 21), and molecular tunnelling opens the route of such polymerisation valid even at $T \rightarrow 0$.

In this way the possibility of energetically profitable formation of unsaturated monomers at the natural balance of elements' abundances would clearly constitute a serious argument in favour of the hypothesis of cold prebiotic evolution in interstellar grains.

(5) When the cloud collapses and a new hot star is created in its centre, the flattened protoplanetary disk formed from the remnants of the cloud continues to be cold. The temperature does not rise during either the consequent accretion of dust particles with the formation of planetesimals (including comets) or the agglomeration of most of planetesimals into the planets²¹⁻²⁷. This makes it possible that the formation of complex polymer molecules in cold interstellar dust can provide a real cold pre-history of life rather than simple accumulation of negative entropy. The molecules formed can reach such a high degree of complexity that they are even able to display the simplest biological functions as soon as the new star begins to heat its own planets and thus sharply increases the rate and variety of chemical reactions.

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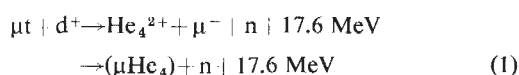
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Muon catalysis of hot fusion

THE idea of muon catalysis of cold fusion dates back nearly 50 years¹⁻³, but the death blow to the technique was given by Jackson⁴. It has been revived by Tan⁵ in connection with fusion in inertially confined pellets and his idea is sufficiently important to warrant further investigation. Our calculations suggest that Tan's estimates are unjustifiably optimistic and so there is no possibility of achieving an energy gain.

The basic reactions are



and the corresponding dd and tt reactions which have a rather

lower probability. We assume, following Tan, that the pellet is totally ionised, and for definiteness consider a 0.5-cm radius pellet at a temperature of 10 keV and solid density. The reactions then occur in flight with a total rate of about 10^9 s^{-1} .

The method avoids the formation of $(\mu\text{He}_4)^+$, which normally terminates the catalysis chain for the following reason: the recoil $(\mu\text{He}_4)^+$ moves off with an energy of 3-4 MeV. Although it is travelling through a dense medium, the usual energy loss mechanism (ionisation of ordinary atoms) is no longer available to it. The only mechanisms available in a plasma are elastic scattering by the Coulomb force and ionisation of the $(\mu\text{He}_4)^+$. Using standard techniques⁶ we find the corresponding rates to be 10^7 s^{-1} and 10^9 s^{-1} (we have rounded off all rates to the nearest order of magnitude). In other words, stripping of the muon is very probable, and it would thus be able to take part in further fusions.

Unfortunately this is irrelevant for the inertial confinement as proposed by Tan. If the confinement time is 10^{-9} s , and the reaction rate 10^9 s^{-1} , on average each muon would catalyse one reaction, hence the occurrence of R_c (the catalytic chain ratio) in equation (5) of ref. 5 is erroneous.

The cost of producing a useful muon (that is, one stopping in the pellet) has been grossly underestimated by Tan. According to Tan e_μ (the energy required to produce one muon) lies between 10^9 and 10^{10} eV , and x_μ (the fraction stopping in the target) between 10^{-4} and 10^{-2} , so that e_μ/x_μ lies between 10^{11} and 10^{14} eV per useful muon. The higher value of e_μ is taken from Jackson who optimistically proposed a ratio of proton beam energy to input electrical power of 0.2 (for TRIUMF, this ratio is ~ 0.01) and assumed that every muon produced was available to catalyse a fusion reaction. In addition, however, only a very small fraction of muons can be focused into a useable beam.

It is more useful in the situation being discussed here to refer to values of e_μ/x_μ that are obtained in today's meson factories, with beams designed to optimise muon stopping rates in small targets. In the standard technique, pions from a target placed in the proton beam are collected in a long ($\sim 10 \text{ m}$) magnetic channel which confines them and their decay muons. The latter emerge from the channel exit in a very crudely focused beam; obviously they cannot be sharply focused as their source is fairly large radially and very long axially. If we take as typical 'state of the art' values, an accelerator input power of $2 \times 10^{25} \text{ eV s}^{-1}$ (TRIUMF: 100 μA at 500 MeV) and a μ stopping rate of $10^5 \text{ g}^{-1} \text{ s}^{-1}$ (SIN: 100 μA at 500 MeV) then for a 0.5-cm radius pellet of density 0.71, e_μ/x_μ is $5.4 \times 10^{21} \text{ eV}$. In fact, as we show below, the stopping rate in an ionised pellet would be very much smaller.

The final objection is the most serious. Whereas it is true that the capture reaction



has a rate of 10^{12} s^{-1} in a solid, it is not true in a plasma, even at solid densities. Using Thomson's classical model⁶, the capture rate is $\sim 10^3 \text{ s}^{-1}$, if we assume the plasma to be totally ionised. In fact, the plasma will be about 99.98% ionised, and the remaining atoms could be reasonably effective in capturing mesons. To estimate this rate, we assume that an atom has the same capture rate in a plasma as would in a solid, and multiply the solid rate by the effective density of atoms. This gives a rate of 10^8 s^{-1} , which in itself is a gross overestimate as capture in a solid occurs only for very slow muons. This is not incompatible with the Saha equation: the only significant population of muons will be in the 1s level, and, by the above arguments, the reaction time for capture into the 1s level is very much longer than the operative timescale of 10^{-9} s .

The same point may be made in slightly different fashion: Tan assumes that the incident muons have an energy of 4 MeV and are stopped in the first 10^{-3} m of the pellet. In fact, the mean free path will be $\sim 80 \text{ cm}$, so if the pellet is pre-ionised, it will be totally transparent to muons. Further, we note that

the pellet will be transparent to the reaction products (4 MeV α 's and 14 MeV neutrons) so no heating of the pellet will occur.

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Water-promoted oxidation of carbon monoxide over tin(IV) oxide-supported palladium

CATALYTIC oxidation of CO, both by O₂ and by nitrogen oxides (NO_x), is of considerable relevance to the removal of toxic CO and/or NO_x from exhaust gases. Certain applications such as automobile exhaust control in cold-start conditions, and the removal of CO from tobacco smoke by use of a catalytic filter, demand not only catalytic activity at moderately low temperatures, but also effectiveness in the presence of water vapour. The low-temperature activities of most catalysts are at best (for example precious metals) partially destroyed, or at worst (for example base metal oxides) totally destroyed by poisoning due to water vapour. The results presented here demonstrate that, not only is the low-temperature activity of the Pd-SnO₂ system not poisoned by water vapour, but rather its presence causes a marked enhancement in activity for the oxidation of CO both by O₂ and by NO.

The catalytic activity of SnO₂-supported palladium for the oxidation of CO both by O₂ (refs 1, 2) and by NO (ref. 3) has previously been shown to be considerably higher than that predicted from purely additive or metal dispersion considerations. This synergism has obvious relevance to potential applications where precious metals are supported on oxide carriers, a typical example being the oxide wash-coats used to 'key' precious metals to the honeycomb monoliths developed for the catalytic control of exhaust gases. These previously reported catalytic data for CO oxidation over Pd-SnO₂ were obtained using anhydrous feed gases, and here we report the effects of adding water vapour.

Samples of 1.8% Pd on SnO₂ and 5.8% Pd on SiO₂ were prepared by non-exhaustive ion-exchange sorption, from Pd(NH₃)₄²⁺ solution, on to 36–72 B.S.S. mesh SnO₂ (ref. 4) and SiO₂ (BDH, pretreated with 1 M HNO₃ and exhaustively washed with water) gels respectively. Thermal activation (5 h at 450 °C in air) and reduction (2 h at 150 °C in 30% CO in Ar) were carried out in the catalytic reactors using anhydrous gases. Specific surface areas after this treatment were 40, 70 and 230 m² g⁻¹ for SnO₂, Pd-SnO₂ and Pd-SiO₂ catalysts respectively. With the exception of the CO-O₂ reaction over Pd-SnO₂ which, because of the low weight required was performed at 60 °C in a 2-mm internal diameter stainless steel U-tube, the previously described⁴ glass reactor assembly was used. Water vapour of various concentrations was admitted to the feed gases by bubbling through H₂SO₄ solutions of

known concentrations. Gas chromatographic analyses of the products of the CO-O₂ and CO-NO reactions have been described (refs 5 and 6 respectively). Catalytic rate data for the CO-O₂ reaction are quoted in terms of CO conversion for a feed of 5% CO in air at 100 cm³ min⁻¹, and those for the CO-NO reaction in terms of NO conversion (both to N₂O and to N₂) for a feed of 14% NO in CO at 30 cm³ min⁻¹.

Typical steady-state (16 h) conversions for the CO-O₂ reaction over the Pd-SnO₂ catalyst (0.25 g) at 60 °C using anhydrous feed gases gave values ranging from 0.7 to 1.2% CO oxidation (four experiments). Introduction of water vapour at a partial pressure of 22 mm Hg caused, after a 4-h conditioning period, an increase in conversion to 10–13%, and this remained constant over a further 24 h period. This order-of-magnitude increase was essentially independent of the water concentration within the partial pressure range of at least 5–22 mm Hg. At p_{H₂O} < 1 mm Hg the enhancement in activity was less than that at higher pressures, but even at p_{H₂O} = 0.1 mm Hg the steady-state activity was still a factor of about 5 higher than the 'anhydrous' rate. Water vapour partial pressures above 22 mm Hg were not investigated because of problems of condensation in the apparatus. The effect of removal of water from the feed gases was to cause only a very slow return to the 'anhydrous' rate. For example, in an experiment in which the 'anhydrous' and 'wet' CO oxidations were 0.7 and 11.7% respectively, removal of the water vapour for 16 h only decreased the conversion to 3.1%. This slow reversibility is presumably attributable to the low rate of dehydration/dehydroxylation of the hydrated surface (see below) at 60 °C.

In contrast to these findings, the steady-state CO oxidation activity of SnO₂ at 170 °C was found to be totally destroyed, and that of the 5.8% Pd-SiO₂ at 140 °C reduced by a factor of >0.2, by the introduction of 22 mm Hg water vapour pressure to the gas feeds.

The presence of water vapour also caused a comparable enhancement in activity for the CO-NO reaction over a 1.0 g bed of the Pd-SnO₂ catalyst at 130 °C. A steady-state (16 h) 'anhydrous' NO conversion of 0% to N₂ and 32% to N₂O was, after the introduction of water vapour at 22 mm Hg partial pressure followed by a 4-h conditioning period, increased to 34% to N₂ and 51% to N₂O. This increased activity remained constant over a further 24 h period although, in contrast to the CO-O₂ reaction at 60 °C, removal of the water vapour resulted in a much more rapid (<16 h) return to the 'anhydrous' rate. This is presumably attributable to a more rapid dehydration/dehydroxylation of the hydrated SnO₂ surface at the higher temperature. In the case of the CO-NO reaction it is apparent that, not only does water vapour increase the overall reaction rate, but it also influences the selectivity towards the preferred (from the pollution control viewpoint) complete reduction of the NO to N₂.

Previous investigations into the CO-O₂ reaction over Pd-SnO₂ have shown strong evidence that the synergistic catalytic effect is attributable to spillover of CO in an activated form from the Pd to the SnO₂ surface². The results presented here suggest that the role of the water vapour is to increase this spillover rate, as the actual catalytic oxidation rates over both pure SnO₂ and over Pd supported on an 'inert' oxide are decreased by the presence of water vapour. A probable explanation for this effect is that water molecules produce hydroxylated sites on the SnO₂ surface which act as chemical bridges to assist spillover of the Pd-activated CO. It has been observed that adsorbed proton acceptors (reported water and alkanols) can assist spillover of activated hydrogen from precious metals on to oxides⁷. But, the mechanism of Pd-activation of CO before its spillover is not yet clear. In the case of hydrogen spillover the activation process

can be readily explained in terms of dissociative chemisorption to produce hydrogen atoms. Even if dissociation were to occur during CO adsorption on Pd, which is unlikely on the basis of current evidence⁸, it is difficult to envisage how this process could explain the above observations.

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Windscale effluent in the waters and sediments of the Minch

A SYSTEMATIC intrusion of water of high ^{137}Cs content into the north-eastern North Sea has been reported by the German Hydrographic Office¹. Jefferies *et al.*² later confirmed that a stream of water rich in radioactive caesium from Windscale passes northwards through the Hebridean Channel, around the north of Scotland and into the North Sea. Although the circulation pattern with respect to ^{137}Cs has been significantly refined in recent reports³ and from German data for 1975 (ref. 4) and 1976 (Kautsky, personal communication) little attention has been paid to the status of other radionuclides in this stream. Such a study offers, however, an excellent opportunity of looking at the geochemical discrimination between, for example, ^{137}Cs and the transuranic α -emitting nuclides; it seemed also that an examination of the changes of the ratio of ^{134}Cs to ^{137}Cs during this passage would give a good basis for estimation of the travel time. As a part of the Flex Programme (Fladen Ground Experiment—part of Jonsdap 76 international programme on the interplay of environment and a plankton bloom) in the North Sea in May–June 1976, we collected a series of sediment, water and biota samples for that purpose. We report here our analyses of samples from a large-volume water station and a 21-cm diameter sediment core taken in the Minch, 27 May 1976.

Our sampling procedures are described elsewhere^{5,6} as are our analytical procedures, and their performance in a variety of IAEA- or NBS-organised intercomparison exercises^{7–10}. Table 1 shows the nuclide analyses so far completed on 55-litre unfiltered water samples from four different depths, together with comparable analyses of alternate sections of the sediment core taken at the same time. For water column and sediment, the inventory inferred for each nuclide from the data presented, and the mean ratios of selected nuclide pairs are shown.

It should be noted that both water and sediment collected anywhere in the oceans today may be expected to contain ^{137}Cs , $^{239,240}\text{Pu}$, ^{238}Pu or ^{241}Am , as a result of their dissemination in world-wide fallout. The analysis of these nuclides and the use of their distributions to infer rates of marine processes are major activities of our laboratory^{11,12}. ^{134}Cs , however, was only a trivial component of world-wide fallout, about $0.25 \times 10^6 \text{ Ci}$ produced¹³, mostly before 1958, compared with $34 \times 10^6 \text{ Ci}$ ^{137}Cs ¹⁴. The ^{134}Cs present in environmental samples collected since 1970 is certainly to be attributed to some aspect of the nuclear fuel cycle. In the case of other nuclides, similar inferences can be drawn either from their presence in amounts far exceeding those to be expected from fallout

delivery measurements, or from departures in their relative proportions from the ratios found in fallout.

The data tabulated indicate Windscale effluent as the preponderant source of the nuclides in the water column and as a major contributor of those in the sediment. This is shown by the large amounts of ^{134}Cs , and by its ratio to ^{137}Cs ; recent data referring to this phenomenon at Windscale have not been published, but the ratio ^{134}Cs to ^{137}Cs approximated 0.23 in 1970², 0.18 in 1971², 0.17 in 1972², 0.19 in 1973¹⁵, 0.24 in 1974³ and 0.18 in 1975³.

Comparison of the water and sediment inventories of ^{137}Cs , ^{90}Sr and $^{239,240}\text{Pu}$ in the Minch location (Table 2) with delivery estimates from fallout at this latitude¹⁶ strongly support the Windscale conclusion in respect of the source of a major fraction of these inventories. A similar conclusion is reached from comparison of the water ratios $^{137}\text{Cs}/^{90}\text{Sr}$ and $^{238}\text{Pu}/^{239,240}\text{Pu}$ with those to be expected at that latitude from fallout. The $^{238}\text{Pu}/^{239,240}\text{Pu}$ ratio of 0.14 is understandable in terms of the reported discharge value from Windscale of about 0.2 for 1971–73 (ref. 17).

Accepting that the major source of the nuclides measured was the Windscale effluent, a variety of hydrographic and geochemical conclusions can be argued. First, from the change in ratio ^{134}Cs to ^{137}Cs in the water, travel time from the Irish Sea can be estimated. ^{134}Cs has a half life of 2.06 yr compared with 30.1 yr for ^{137}Cs . Making the assumption that the two caesium isotopes are not discriminated in ocean circulation, and less satisfactorily that a ratio of 0.24 characterised the source term, then from the ratio 0.144 observed at the Minch it is concluded that the travel time from the Windscale pipeline to the Minch was about 1.6 yr. If source term ratio had been closer to 0.18, as might be argued from other data^{2,3}, then a travel time closer to 0.7 yr would be concluded. Neither calculation is at first sight compatible with the very slow passage times that were suggested by Jefferies *et al.*² for transit from Windscale to the North Channel. An appreciable fraction of the total travel time may have been spent in the Irish Sea, and this, in fact, indicated by the mean ratio 0.20 reported³ in place and *Nephrops* from the northern Irish Sea in 1974. In the Irish Sea, reduction of the ^{134}Cs to ^{137}Cs ratio by mixing with older, lower ratio water from Windscale could be significant, especially considering the ratios reported for 1971–1973 releases; reduction by mixing with fallout ^{137}Cs free of ^{134}Cs is suggested to be insignificant by the expected fallout ^{137}Cs concentration in coastal water, about 0.2 pCi l^{-1} at most by 1973–1974¹¹ and little more than 0.1 by 1976 (Kautsky, unpublished).

The most likely solution to the dilemma caused by the different transit times derived from this and from Jefferies' work² is that we are seeing the net effect of two (at least) processes. First, the mixing process within the Irish Sea and, second, the outflow from the Irish Sea into the coastal current travelling northwards parallel to the west coast of Scotland. The first process may be very much slower than the second so that the net effect may be controlled more by the mixing times within the Irish Sea. Additional support for this two-component process comes from comparison of our ^{137}Cs concentrations in the Minch in May 1976 with the values about $4\text{--}5 \text{ pCi kg}^{-1}$ for July 1974³. Our values of $4.7\text{--}6.5 \text{ pCi kg}^{-1}$ are not significantly different from the values measured 1.8 yr earlier. As these show little difference, it is arguable that May 1976 samples from the Minch have not begun to be affected by the rise in Windscale ^{137}Cs discharge described by Hetherington³ in 1974. So a net transit time of 2 yr could be composed of a term of 1.8 yr in the Irish Sea and 0.2 yr in the coastal current between North Channel and the Minch. Additional evidence that the coastal current labelled with radionuclides originating from Windscale is moving rapidly, comes from preliminary measurements of the $^{134}\text{Cs}/^{137}\text{Cs}$ ratio in water, also collected in May 1976, in the North Sea between Scotland and Norway. At this location (58°25.5'N, 0°03.4'E), the measured ratio of 0.14 is indistinguishable from the ratios measured in the Minch.

Second, from comparison of the inventory of radiocaesium in the sediments of the Minch against the estimated total delivered to the area, with the same relation in respect to fallout, some

Table 1 Radionuclide analyses of water and sediment from the Minch

RV Knorr cruise 54 Leg 6 Station 84: 27 May 1976 at 58° 14.6'N; 05° 49.5'W						
Water station	$^{137}\text{Cs}^*$	$^{134}\text{Cs}^*$	$^{90}\text{Sr}^\dagger$	$^{239,240}\text{Pu}^\ddagger$	$^{238}\text{Pu}^\ddagger$	$^{241}\text{Am}^\S$
Sample depth (m)	(pCi l ⁻¹)	(pCi l ⁻¹)	(pCi l ⁻¹)	(fCi l ⁻¹)	(fCi l ⁻¹)	(fCi l ⁻¹)
Surface	6.13 ± 0.03	0.88 ± 0.05	0.998 ± 0.009	2.6 ± 0.2	0.28 ± 0.09	0.32 ± 0.09
30	6.52 ± 0.05	0.92 ± 0.04	1.00 ± 0.02	2.6 ± 0.1	0.41 ± 0.05	0.09 ± 0.09
60	5.84 ± 0.04	0.86 ± 0.02	0.923 ± 0.009	2.6 ± 0.2	0.46 ± 0.09	0.46 ± 0.09
90	4.66 ± 0.04	0.66 ± 0.01	0.793 ± 0.023	2.2 ± 0.1	0.28 ± 0.05	0.32 ± 0.09
Water depth (100 m)						
Water column inventory (mCi km ⁻²)	579	83.1	92.5	0.25	0.038	0.029
Mean nuclide activity ratios	$^{134}\text{Cs}; ^{137}\text{Cs}, 0.14^\ddagger; ^{137}\text{Cs}; ^{90}\text{Sr}, 6.2; ^{238}\text{Pu}; ^{239,240}\text{Pu}, 0.14; ^{241}\text{Am}; ^{239,240}\text{Pu}, 0.12$					
RV Knorr cruise 54 Leg 6 CORE 46: 27 May 1976 at 58° 06.1'N; 05° 51.0'W						
Sediment core	$^{137}\text{Cs}^*$	$^{134}\text{Cs}^*$	$^{239,240}\text{Pu}^\ddagger$	$^{238}\text{Pu}^\ddagger$	$^{241}\text{Am}^\S$	
Sediment section (cm)	(pCi kg ⁻¹)	(pCi kg ⁻¹)	(pCi kg ⁻¹)	(pCi kg ⁻¹)	(pCi kg ⁻¹)	(pCi kg ⁻¹)
0-1	748 ± 18	65 ± 11	37 ± 1.6	3.4 ± 0.3	12.5 ± 0.5	
2-3	536 ± 9	44 ± 2	31 ± 1	2.8 ± 0.2		
4-6	518 ± 14	30 ± 7	25 ± 1.2	1.7 ± 0.2		
8-10	232 ± 9	26 ± 5	17 ± 0.9	0.65 ± 0.09		
12-14	86 ± 4	1.7 ± 1.3	5.7 ± 0.3	0.30 ± 0.05		
16-18			0.4 ± 0.5	0.06 ± 0.02		
Water depth (91 m)						
Sediment column inventory: (mCi km ⁻²)	55.3	4.09	3.1	0.21		
Mean nuclide activity ratios	$^{134}\text{Cs}; ^{137}\text{Cs}, 0.074; ^{238}\text{Pu}; ^{239,240}\text{Pu}, 0.069$					

Where values are not given the analyses are incomplete.

*By Ge (Li) γ spectrometry after radiochemical separation.

†By anticoincidence β counting after radiochemical separation.

‡By α spectrometry after radiochemical separation.

§By α spectrometry after radiochemical separation; curium isotopes accompany Am in our procedure. No ^{242}Cm was observed, and ^{244}Cm was present only in the amount (about 1 count per day) added as an impurity in our ^{243}Am yield monitor.

conclusions can be drawn about the geochemical behaviour of Windscale radiocaesium. Noshkin and Bowen¹⁶ reported about 7% of fallout ^{137}Cs delivery was found in very shallow-water sediments of Buzzards Bay; using more recent data to interpolate between Buzzards Bay and the 200-m cores from slightly further north, one might predict only 5–6% of fallout in sediment cores under 100 m of water.

Estimating the Windscale-derived delivery of Cs nuclides to the Minch is difficult at best, and made even more so by the fact that Windscale release data for ^{137}Cs have been published only for the years 1957–67¹⁴ and 1972–73¹⁷. Using the data available, and extrapolating to estimate an annual average, ^{137}Cs release of 15,000 Ci for 1968–71, yields a release estimate, through 1973, of 156 kCi, which, divided by the area of the Irish Sea (after Hetherington¹⁷), corresponds to a ^{137}Cs delivery of 6.2 Ci km⁻². The fraction of Windscale ^{137}Cs reaching the Minch can be estimated by comparing the water column inventory (or the total inventory, water plus sediment) in Table 1 (579 or 634 mCi km⁻²) with the mean Area A water column inventory of 2.40 Ci km⁻² in 1974¹⁷. This comparison is consistent with the 2 yr travel time, from Windscale to the Minch, that we inferred above from the ^{134}Cs to ^{137}Cs ratio and ^{137}Cs concentration data. If the shorter, 0.7 yr, travel time were correct, then the inventory for comparison should be that, in the Irish Sea, of late 1975, requiring use of data

not yet available. One arrives, therefore, at the conclusion that the Minch 'sees' about 25% of the ^{137}Cs delivered by Windscale to the Irish Sea, and this should have represented, by 1976, at least 1,550 mCi km⁻² ^{137}Cs delivery to the Minch, and possibly more. The 3.5%, or less, of the inferred Minch delivery of ^{137}Cs found in the sediments may differ significantly from the 5–6% predicted for fallout ^{137}Cs , but we do not believe that the quality of the estimated values justifies this conclusion. If we were to take it seriously, we would argue that it shows either a systematic difference in properties of the Minch sediments, or the effect of isotope exchange at the much higher ^{137}Cs specific activities found in Windscale effluent. Certainly the observations do not suggest the Windscale caesium as either more or less reactive than that from fallout.

Third, from comparison of the ratio of the water column inventories of ^{137}Cs and $^{239,240}\text{Pu}$ with their ratio in the north-eastern Irish Sea conclusions can be drawn about the long-range trajectory of Windscale plutonium in British coastal waters. Calculated inventories of soluble $^{239,240}\text{Pu}$, and of ^{137}Cs , in the waters of the Irish Sea are available¹⁷ for both 1973 and 1974. From these it seems the mean ratio $^{239,240}\text{Pu}$ to ^{137}Cs in July 1974 was 0.094%. This figure is probably representative of the Irish Sea ratio at this time as it was shown not to vary between points within 10 km of the Windscale outfall and points 75–100 km from the

Table 2 Comparison of Minch nuclide inventories and ratios with fallout

	^{137}Cs	Inventories ^{90}Sr (mCi km ⁻²)	$^{239,240}\text{Pu}$	$^{137}\text{Cs}/^{90}\text{Sr}$	Ratios $^{238}\text{Pu}/^{239,240}\text{Pu}$
Water	—	93	—	6.2	0.14
Water plus sediment	630	—	3.3	—	—
Fallout	80	55	1.5	1.0	0.04

outfall. It differs significantly from the ratio, 0.043%, observed in 1976 in the water column of the Minch. If the 1976 Minch water was the same water mass as characterised the Irish Sea in July 1974, one could conclude from the $^{239,240}\text{Pu}/^{137}\text{Cs}$ ratio change that only 46% of the soluble plutonium that was associated with the ^{137}Cs transported by the coastal current, survived the passage to the Minch. This conclusion, that rather less than half as much of the soluble plutonium avoids sediment uptake during transit from the Irish Sea to the Minch, implies that Windscale 'soluble' plutonium is only partly 'qualitatively similar'¹⁸ to ^{137}Cs . That the details of the separation of these two species may be of predictive importance is indicated by our preliminary data concerning sediments from the Flex area, of the North Sea, where ^{238}Pu to $^{239,240}\text{Pu}$ ratios, as do those of ^{134}Cs to ^{137}Cs , indicate significant delivery of Windscale radionuclides.

Fourth, from comparison of the ratio of ^{241}Am to $^{239,240}\text{Pu}$ observed in the Minch, against that reported in releases from Windscale¹⁸, some conclusions can be drawn about the relative mobilities of these two transuranium elements in marine coastal waters. Hetherington *et al.*¹⁸ have reported the mean ratio ^{241}Am to $^{238,239,240}\text{Pu}$ in Windscale releases to have been 2.56 in 1974, 1.66 in 1973 and 1.40 in 1972; it has also been reported¹⁹ that there were very wide fluctuations in this ratio, month to month, in 1974 releases. The mean ratio observed in the water of the Minch, 0.12, confirms that a very much greater proportion of ^{241}Am than of plutonium is immobilised during travel in the coastal current. A similar conclusion can be drawn from the low $^{241}\text{Am}/^{239,240}\text{Pu}$ ratios recently reported by Murray⁴ in seawater collected in 1975 from around the Orkney Islands. Although reports have consistently shown^{3,15} high ratios of ^{241}Am to plutonium in attached algae like *Porphyra*, it is likely that the major process immobilising ^{241}Am is association with sediments. This is indicated by the ratio of 0.33 observed in the surface sediments of the Minch, compared with the 0.12 observed in the overlying water. Some of the difference in the two ratios may be attributed to ingrowth of ^{241}Am from its parent ^{241}Pu (as discussed in refs 18, 20) but we believe most of the effect should be attributed to separation of americium and plutonium in the water column. We have described elsewhere²¹ differential behaviour of americium against plutonium from fallout in oceanic water columns. We submit that Hetherington *et al.*¹⁸ were unable to see this differential behaviour in the Irish Sea because of the fluctuations in supply ratio, and because trajectories were too short for the differences to be easily measurable.

Analyses of long-lived radionuclides in a water station and sediment core from the Minch have, therefore, confirmed the presence of Windscale-produced ^{90}Sr , ^{134}Cs , ^{137}Cs , ^{238}Pu , $^{239,240}\text{Pu}$ and ^{241}Am . Careful examination of the evidence for changes in the various ratios of these nuclides from those reported in the Windscale release stream has also (1) supported an improved estimate of the northward rate of movement of these nuclides in the coastal current; (2) confirmed that Windscale caesium behaves biogeochemically about as does that from fallout; (3) indicated that the soluble fraction of Windscale plutonium may qualitatively behave like ^{137}Cs , but that only some fraction of it survives travel to the Minch, relative to ^{137}Cs . The size of this fraction, put at about one-half from these measurements, and its possible variation in space and time should be determined; (4) demonstrated a large differential in the behaviour of ^{241}Am and $^{239,240}\text{Pu}$, in that americium seems to sediment out of the coastal current even sooner than does plutonium after introduction from Windscale.

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Surge activity on the Barnes Ice Cap

THERE are conflicting opinions¹⁻⁸ on the occurrence of surges in the major ice sheets, deduced in many cases by indirect means. Where an ice sheet surges directly into the ocean, sea-level rise may be catastrophic. Surges in ice sheets may help to explain the apparent difficulties of accounting for the magnitudes of the decay rates deduced for the Laurentide Ice Sheet in terms of energy requirements for melting ice⁹. That is, large areas of that ice sheet became much thinner than was supposed, a suggestion made on the basis of marine core evidence¹. Pure computer simulations¹⁰ have shown that cyclic surging of the major ice sheets seems to be possible. This report shows that several surges have occurred within the last millennium on the Barnes Ice Cap, a small subpolar ice sheet on Baffin Island, Canada (Fig. 1).

This ice cap is a remnant of the North American Laurentide Ice Sheet¹¹. Surge scars on the ice cap have been tentatively identified¹² and one surge area was subsequently documented in some detail¹³. Now, five distinct surge scars of different ages have been identified with the help of Landsat imagery (Fig. 2). The scars all lie on the south-west side of the ice cap, which is thought to be significant because the south-west margin of the ice cap is, on average, 130 m lower in altitude than the north-east margin and because the orientation of the axis of the ice cap causes about 5% more direct solar radiation to be received on the south-west facing slopes¹⁴. Therefore, the average bulk temperature of the ice on the south-west side should be higher than that of the north-east side. The attainment of basal conditions favourable to surges should thus be expected first on the south-west side.

Except where immediately adjacent to proglacial lakes, the ice margin is frozen to the bed^{15,16}. Measurements (D. F. Classen, unpublished) indicate that ice at the pressure melting point exists at the base in the interior (Fig. 3) and approximate calculations¹³ indicate that this condition could be widespread. Horizontal velocity measurements (Fig. 3) tend to confirm this deduction since considerable basal sliding must be present.

Conn and Bieler Lakes are evidently formed as a result of the ice cap obstructing water flow along the natural slope of land. Generator and Blanchfield lakes have been formed by relatively recent surge ice which has interfered with previous drainage away from the ice cap. The presence of these lakes tends to accelerate ice flows at the margins¹⁵ compared with locations without lakes^{16,17}. Thus, part of surge areas numbers 1 and 3 may be suffering delayed 'recoveries' because of the proglacial lakes.

The general features of the surge scars are: (1) the presence of third and occasionally fourth order meltwater channels. These occur within the large surface depressions characterised by highly irregular topography not related to bedrock topography. The depressions displace the ice divide, and the corresponding bulged margins often extend well beyond the

¹ *Umweltradioaktiv. Strahlenbelast.* **51**, (1971); **52**, (1972); **64**, (1973).

² Jeffries, D. F. *et al. Mar. Pollut. Bull.* **4**, 118-123 (1973).

³ Hetherington, J. A. *Rep. FRI 11* (1976); **12** (1977) (Fisheries Radiobiology Laboratory, Lowestoft).

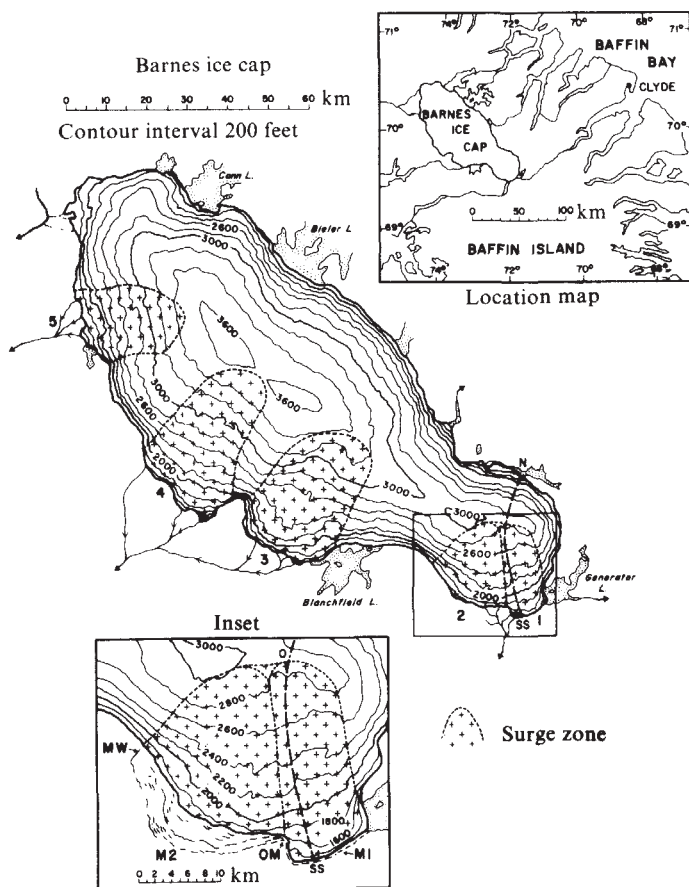


Fig. 1 Map of Barnes Ice Cap showing locations of former ice surges. Inset of south dome area shows location of transect along which data are presented in Fig. 3. Note moraine ridges (MW, M2, OM) associated with surge area no. 2. M1 marks recent moraine adjacent to surge area no. 1.

average line of the ice edge (Fig. 2). (2) Irregular foliation, defined by alternate dark- and light-banded ice (Fig. 2) is developed near the margins¹⁶ and indicates the presence of large shear strains. Measurements¹⁷ in non-surged areas show that the horizontal component of the ice flow vector is roughly parallel to the maximum principal strain rate direction (implying small shear strains) whereas within surge area no. 1, divergence between these quantities reaches 40° (G.H., unpublished). (3) Lakes occur on the ice surface due to trapping of meltwater in local depressions. While many of these lakes are visible on 1961 aerial photographs, some are visible on Landsat imagery

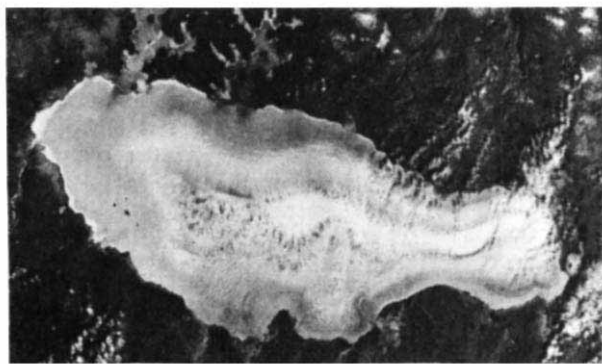


Fig. 2 Landsat image of Barnes Ice Cap (Channel 7, August 1974) showing surge scars. Surge areas 1 and 2 are partly obscured by cloud. Note evidence of intense shear strain in lower part of surge area no. 3 where ice is banded and the existence of lakes in surge area no. 5 (dark patches). The crown of the ice cap is covered by snow (white area) and meltwater streams cause the streak patterns.

(Fig. 2). Several moulins traversing cold ice are present at the margins. (4) Major streams, which flow west from the ice cap, can all be traced to the major surge areas (Fig. 1). (5) Moraines adjacent to the surge lobes tend to be well developed particularly when the surge is recent, since the moraines initially contain a substantial ice core. Also, recently overridden and pushed sediments may be seen near SS in Fig. 1. Ice depth soundings (J. W. Clough, unpublished and refs 18, 19) in surge zones 1,

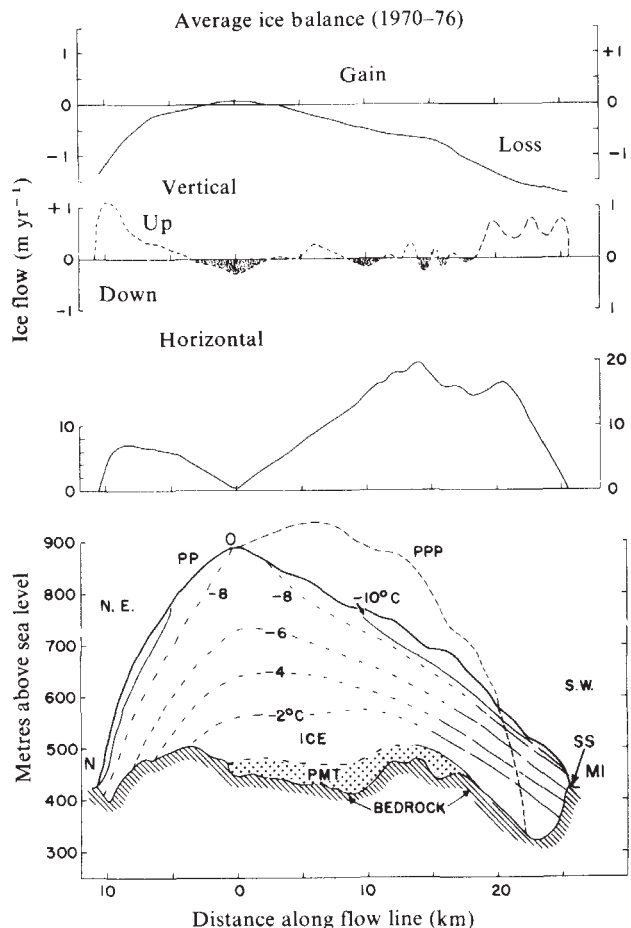


Fig. 3 Cross section through south dome from north-east to south (see Fig. 1) showing ice thickness, temperature, surface flow rates and net mass balance. Note relative irregularity of measured quantities on south (surge) section compared with the same quantities on the north-east section. PP, present profile; PPP, postulated pre-surge profile. Isotherms are shown in short-dashed lines where values have been estimated and long-dashed lines where measurements have been obtained.

2 and 3 show that there, the surface profile does not owe its shape to the basal topography in terms of standard flow theory²⁰. These profiles cannot be fitted to established equilibrium ice-cap profiles²⁰ whereas the contiguous profiles on the north-east side can^{13,17}. Profile PP (Fig. 3) is taken through O-N and O-SS-flowlines (Fig. 1). Plotted isotherms are based on measured values and approximate computation¹⁰. As this is not a steady state situation, the correct solution can only be found by successfully modelling the complete surge or by total measurement. Nevertheless, at least some, and possibly a substantial part, of the base may be at the pressure melting point, switching to cold base at about 18 km down flowline O-SS. Profile PPP is an hypothetical pre-surge parabolic profile which would seem to satisfy mass conservation after allowing for long term mass balance¹⁰. Recent surface net ice balance is also plotted, and comparing this with vertical ice flow values (Fig. 3) it can be seen that, whereas the north-east side is approximately near equilibrium, the south-west side is rapidly wasting. Furthermore,

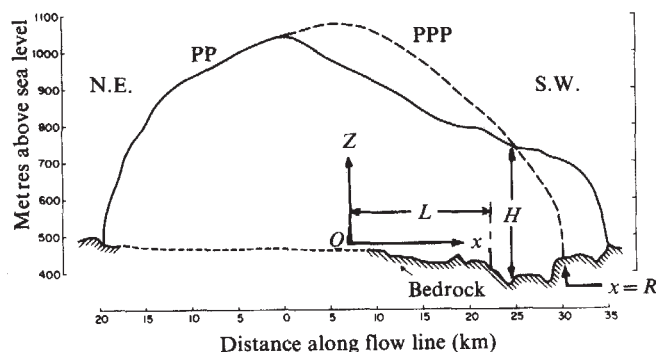


Fig. 4 Cross section through ice cap along two opposing flow lines. The south-west flow line is along the centreline of surge area no. 3. Coordinates and dimensions for equation developed in the text are shown. PP and PPP are as in Fig. 3.

the wavy character of the vertical ice flow on the south-west side indicates a disturbed dynamic flow regime.

This surge could have occurred as recently as 1927 or just before the turn of the century depending on the mass balance¹³, interpretation of the moraine ages²¹, and on the credibility of expedition accounts by Inuit from Clyde (Fig. 1).

Surge no. 2 is associated with a significant frontal moraine system (Fig. 1: inset, Fig. 2 and ref. 21) which may be interpreted in terms of cyclical surge activity with a period of 300–400 yr, spanning a period warmer than today and the 'Little Ice Age'. Morainial geometry indicates that local ice-cap dynamics was involved in their formation. The outer moraine curves sharply towards the ice cap at the north-west end (MW in Fig. 1: inset); at the eastern end (OM) it is truncated by the no. 1 surge lobe. The general retreat of the ice margin (M2) after about AD 500 (J. T. Andrews, personal communication) was punctuated by events at ~AD 900, ~AD 1200, ~AD 1500 and ~AD 1900 (or contemporaneously with surge no. 1)²¹.

Surge no. 3 may have reached a maximum position in about AD 1700 when it caused the formation of Blanchfield Lake in a more expanded form¹⁸. Area no. 4 has similar features to no. 3 and may be of comparable age whereas area no. 5 may be much older. The surface contains low intensity foliation and several large lakes. The inferred cyclic surging of diminishing extent seen for surge area no. 2 may or may not have been present in the other areas, but is a mechanism for maintaining a long-term surge scar.

Study of the surge mechanism is hindered by the lack of data that apply before and during the early stages of the surge. But it seems reasonable to assume that the mechanism described by Schytt²² applies. Figure 4 shows the profile through surge area no. 3 (PP). Let the parabolic pre-surge profile PPP be defined by ice thickness $H(x)$ and radius R of which only an outer portion $R-L$ is frozen to the bedrock. The length L ($< R$) is supposed to grow during a surge build-up. The amount of interior ice at the pressure melting point is thought to be controlled by heating of the upper surface as a result of superimposed ice build-up at the surface over sufficient period of time (10^3 – 10^4 yr). In this way, the onset of surging is related to climate.

As the water layer, represented by L , spreads, more interior ice is able to slide and because of its higher temperature, it will creep faster than the cold marginal ice. A predominantly mechanical mode of failure of the ice dam may follow when

$$\int_0^H \sigma_{xx} dz = \int_L^R \sigma_{xz}^b dx$$

where $\sigma_{xx}(z)$ is the horizontal component of the stress tensor at $x = L$ and $\sigma_{xz}^b(x)$ is the basal shear stress $L < x < R$. The

catastrophic flow which ensues might best be modelled using the finite element method incorporating joint elements (T. Q. Nguyen, unpublished).

A similar process may have occurred in the decaying phases of the Laurentide Ice Sheet in which surges have been inferred from the geological record⁸.

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The rise and fall of intracellular pH of sea urchin eggs after fertilisation

JOHNSON *et al.*¹ have reported an increase in the intracellular pH of sea urchin eggs, seen between 1 and 4 min after insemination, that results from the efflux of H^+ ions from the cells. The rise in pH can be induced by artificial activators of metabolism such as ammonia, or organic amines such as procaine. They have postulated that this increase in cellular pH is responsible for the metabolic derepression of the eggs and the initiation of embryonic development^{1,2}. Our interest in the control of the initiation of DNA synthesis in these synchronously dividing cells³, coupled with their method¹ for estimating the intracellular pH, led us to attempt to determine if fluctuations in pH occur during the cell cycle. Here we confirm the important observation of a rise in pH at fertilisation, and we report that the rise is soon followed by a gradual drop in pH to final values lower than those of the unfertilised condition.

The pH of homogenates of unfertilised sea urchin eggs averaged 6.34 ± 0.1 (41 determinations). By 10 min after insemination the pH rises to an average of 6.76 ± 0.16 (five determinations, Fig. 1). These values are in close agreement with those of 6.48 (unfertilised) and 6.76 (10 min after insemination) reported by Johnson *et al.*¹ with the exception that their average difference between unfertilised and fertilised of 0.28 is smaller than ours of 0.43 units. After a peak rise at 10 min after insemination (Fig. 1), the pH steadily decreases, reaching the value of the unfertilised eggs (6.46) by 70 min and attaining a final value of 6.18 by 140 min, a value that is maintained to at least the 48 h, mid-gastrula stage. No correlation of pH with stage of the cell cycle was found. Identical results were obtained when unfertilised eggs were parthenogenetically activated with the Ca^{2+} ionophore A23187 (ref. 4). It can therefore be concluded that although the initial rise in pH may be obligatory to metabolic activation, maintenance of elevated intracellular pH is not necessary to sustain embryonic development.

Does measurement of the pH of egg homogenates give a valid estimate of intracellular pH? The possibility that

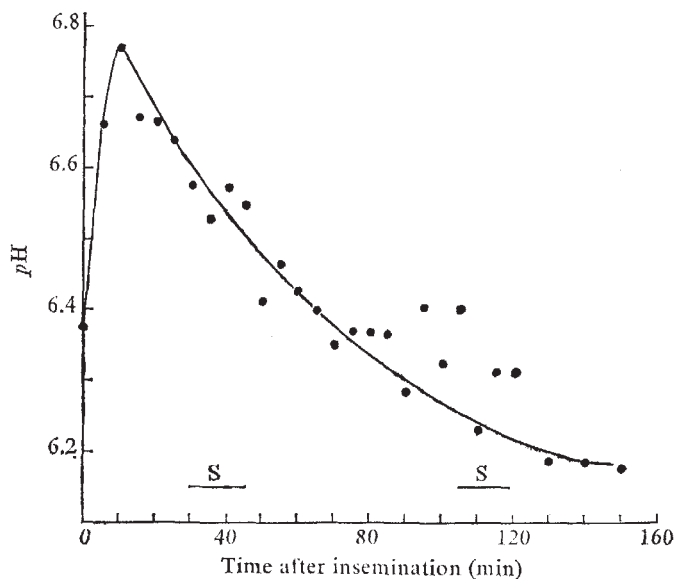


Fig. 1 The pH of sea urchin egg homogenates plotted against time after insemination (average of five separate experiments). Eggs of *Strongylocentrotus purpuratus* were stirred in seawater (pH 7.8) at 1% (v/v) concentration in a 15 °C room in which all media were stored and all pH determinations were accomplished. Aliquots of 50 ml of the culture were sedimented in a 50-ml conical centrifuge tube by hand centrifugation. The seawater was removed and the eggs suspended in 30 ml of bicarbonate-free artificial seawater, pH 7.8 (ref. 1). After resedimentation and removal of the artificial seawater the eggs were resuspended in 5 ml of 0.55 M KCl adjusted to pH 7.0 with HCl and NaOH. The suspension was transferred to a 20-ml Dounce homogeniser and 10 passes were made by hand with a tightly fitting Teflon pestle. Microscopic examination showed that no intact eggs or egg fragments remained. The pH was measured immediately with an expanded scale Corning Model 10 pH meter equipped with a Thomas 4094-L15 universal combination electrode. The timing of the first two periods of DNA synthesis³ is denoted by S on the horizontal axis. The protein concentration of the homogenate was 11.6 mg ml⁻¹. Homogenates containing one half or twice as much protein did not show significant pH differences.

the rise and fall in pH might not be a true estimate of intracellular pH but merely a reflection of compartmentation of H⁺ or OH⁻ ions in organelles such as mitochondria, was investigated by destroying all membrane-enclosed compartments by homogenisation in 0.5% Triton X-100. The results of this experiment (Table 1) show that the rise and fall in pH was also obtained in Triton, suggesting the method might prove to be valid for estimating total cellular pH of these and of other types of cells.

Fertilisation results in an irreversible activation of synchronous cycles of DNA synthesis and cell division³. We previously showed that DNA synthesis in unfertilised sea urchin eggs can be reversibly initiated by treatment with the local anaesthetic procaine hydrochloride⁵. If procaine is removed when the cells are in mid S phase, DNA synthesis abruptly halts. Other workers have shown that procaine treatment causes the efflux of H⁺ ions from these cells which results in a rise in intracellular pH; that is, the weakly basic nature of procaine is not responsible for the observed rise in cellular pH^{1,6}. We wondered if the removal of procaine from eggs would result in a

decrease in intracellular pH which might serve, somehow, as a switch to turn off DNA synthesis. We repeated our earlier experiments of treating unfertilised eggs with 10 mM procaine in seawater for 75 min, then washing out the drug, reculturing in fresh seawater, and determining the pH of egg homogenates. On addition of procaine the intracellular pH rises sharply and remains high for the duration of procaine treatment (Fig. 2). When procaine was washed out and the eggs returned to normal seawater, the intracellular pH rapidly dropped to the value of the untreated eggs, pH 6.46 (Fig. 2).

After their peak rise at 10 min after insemination, fertilised eggs (Fig. 1) required 60 min to return to their unfertilised pH. After procaine removal, however, unfertilised eggs required only 20 min to drop to their original pH (Fig. 2). This difference in rate of pH drop suggests that different mechanisms may underlie the fall in pH fertilised eggs and in unfertilised eggs recovering from procaine treatment. Support for this hypothesis was found in experiments in which fertilised eggs and procaine-activated eggs were treated with the metabolic poison dinitrophenol (DNP). For example, eggs were pretreated with DNP 30 min before fertilisation or procaine addition. Because DNP kills sperm, the eggs to be fertilised were washed from DNP into seawater (5 min washing time) and sperm immediately added. DNP was then added again 2 min after insemination. The normal rise in pH occurred, peaking at 10 min (Fig. 3), but the greater part of the subsequent drop in pH did not occur, and the cells maintained a pH of 6.65 for the duration of the experiment. Identical results were obtained when eggs were parthenogenetically activated by the ionophore A23187 in the continued presence of DNP. In response to procaine, the pH of DNP-treated, unfertilised eggs rose to 6.90 and remained at that value until 75 min when the procaine was washed out with seawater containing DNP. The result was an almost immediate drop in pH back to the original value (Fig. 3). The rise in intracellular

Fig. 2 Intracellular pH and the addition and removal of procaine. Procaine hydrochloride (10 mM, Sigma) was added to a suspension of unfertilised eggs and a rapid rise in the intracellular pH immediately occurred which was sustained for the duration of procaine treatment. At 75 min the procaine was removed (arrow) and the eggs returned to normal seawater⁵. There was a rapid fall in intracellular pH to the untreated value. To be certain that residual procaine was not influencing the pH of homogenates we added 0.5 ml 10 mM procaine to homogenates and found that it did not change the observed pH.

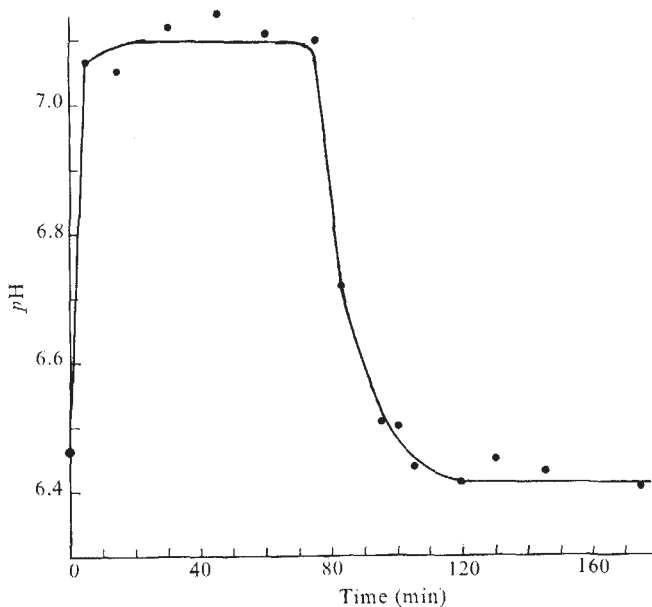


Table 1 The pH of egg homogenates in 0.55 M KCl containing 0.5% Triton X-100

Time after insemination (min)	pH
0	6.39
10	6.70
60	6.58
120	6.43

pH between 0 and 10 min after insemination or after procaine addition in the presence of DNP is evidence that H^+ ion release from mitochondria is probably not responsible for excretion of H^+ from the cells. The subsequent fall in pH in fertilised eggs may depend on the synthesis of ATP (with the reservation that metabolic inhibitors can have nonspecific effects). The drop in pH of the unfertilised eggs immediately after procaine removal in the presence of DNP shows that in these eggs the drop is independent of the production of metabolic energy.

The normal drop in intracellular pH of fertilised eggs (Fig. 1) is the most protracted metabolic change yet reported in the metabolic activation of development², a final value not being reached until 140 min after insemination. This is well after the completion of the first mitosis and the second S phase³. The period of the pH drop corresponds to the period of the activation of the transport systems for amino acids^{7,8} and nucleosides⁹ and the increase in the rate of protein synthesis⁷ in these cells. It is tempting to speculate that the development of transport capabilities might be linked to the lowering of intracellular pH. In this context, we have reported that the transport of thymidine into unfertilised eggs treated with procaine is only 2% that of fertilised eggs, whereas DNA synthesis in procaine occurs at roughly 50% the rate of fertilised eggs³. The high intracellular pH maintained in procaine may somehow inhibit the development of the transport systems in the cell membranes.

DNA synthesis is reversibly activated by the addition and removal of procaine³. The rapid drop in pH after procaine removal and the insensitivity of the drop to respiratory poisons such as DNP show that procaine may activate DNA synthesis by a different mechanism than does fertilisation. Amphipathic drugs such as procaine are thought to affect cells by intercalating into the lipid bilayers of the cell membrane. This can cause the expansion of the membrane which may result in a change in its permeability characteristics¹⁰. Procaine may open channels for H^+ ion excretion which would result in an elevation

of the intracellular pH. After removal of procaine from the external medium, procaine loss from the membrane may re-establish normal permeability, thus allowing the cells to return to a lower pH. The rise and fall in pH on the addition and removal of procaine correlates well with the turning on and turning off of DNA synthesis⁵ indicating that, in these conditions of artificial activation, the control of DNA synthesis may indeed be linked to changes in intracellular pH.

We thank David W. Deamer and Robert J. Gillies for helpful discussions. This work was supported by the USNIH.

Note added in proof: Nishioka and Epel using the homogenisation method, and Shen and Steinhardt using pH microelectrodes have confirmed the transient rise in pH of sea urchin eggs after fertilisation. The pH values obtained by both these groups are slightly higher than those we report here.

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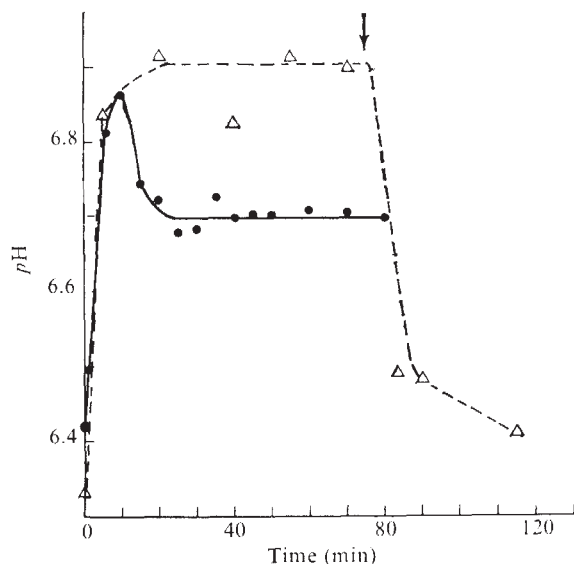
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Calcium uptake during mitosis in the myxomycete *Physarum polycephalum*

THE notion that Ca^{2+} is involved in the regulation of the kinetic and contractile events associated with mitosis in eukaryotic cells is attractive. The contractile proteins, actin and myosin, have been identified in nuclei of *Physarum polycephalum*¹ and their interaction is apparently regulated by Ca^{2+} (ref. 2). The formation of spindle microtubules may also be sensitive to Ca^{2+} (ref. 3), although as yet there is little evidence of Ca^{2+} fluctuations during mitosis. Macropasmodia of the myxomycete *P. polycephalum* are particularly suited to the analysis of events during the cell cycle and mitosis. Synchronous mitoses of large numbers of nuclei proceed against a lowered background of contractile events in the surrounding cytoplasm as cytokinesis does not occur and cyclosis or cytoplasmic streaming slows or ceases during the mitotic period⁴. Extranuclear filaments form in telophase⁵, presumably to participate in nuclear division, but earlier nuclear mitotic events should be amplified. We present evidence here that cyclic uptake and release of Ca^{2+} from the extracellular medium occurs during mitosis in *P. polycephalum*, and that these fluctuations correlate with specific structural and kinetic events in the mitotic nuclei.

Plasmodial Ca^{2+} uptake was monitored by adding $^{45}Ca^{2+}$ to the medium, removing samples of plasmodia at 1-min intervals and assaying the radioactivity accumulated. During the interphase period before mitosis, a rapid and regular cyclic uptake and release of $^{45}Ca^{2+}$ is seen (Fig. 1). The frequency of this process is similar to oscillations in light absorption measured in a small plasmodial segment (Fig. 1 inset) which Sachsenmaier *et al.*⁴ correlated with shuttle streaming. Similar observations on cyclical variation in free cytoplasmic Ca^{2+} concentrations during shuttle streaming in *Physarum* have been made by Ridgway and Durham¹⁰. During mitosis a different pattern of Ca^{2+} uptake is observed (Fig. 1). Although some fluctuations in uptake still occur, the regular periodicity detected in interphase is not apparent. In metaphase there is an outflow of more than half of the $^{45}Ca^{2+}$ accumulated during prophase and uptake is substantially reduced for 3–4 min. During

Fig. 3 Effect of dinitrophenol on the fall in pH of fertilised eggs and procaine-activated eggs. Unfertilised eggs were treated for 30 min with 2.5×10^{-4} M DNP in seawater. Eggs to be fertilised were quickly washed from DNP into seawater (the wash required 5 min), sperm was added and 2 min later DNP was reintroduced. The normal rise in pH occurred but the subsequent drop in pH was blocked. Procaine was added directly to the DNP-treated unfertilised eggs and the elevation of pH immediately occurred. At 75 min the procaine was washed out with seawater containing DNP. The intracellular pH rapidly dropped to the value close to that of untreated eggs. ●, Fertilised eggs; △, procaine-treated unfertilised eggs.



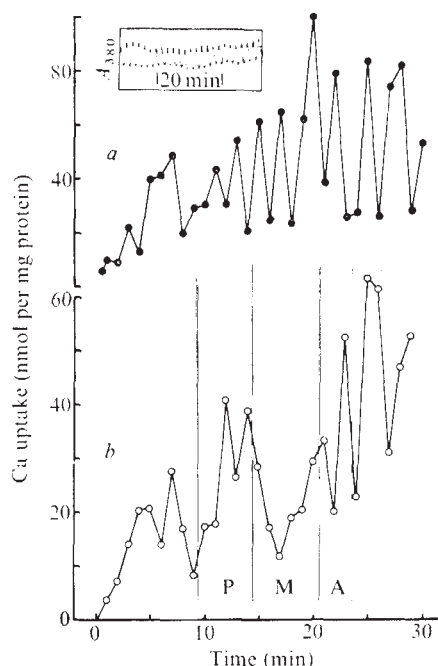


Fig. 1 Time course of $^{45}\text{Ca}^{2+}$ uptake during interphase and mitosis. Macroplasmodia were initiated and cultivated⁶ on filter paper disks at 25 °C using a semi-defined medium⁷ (EDTA, thiamine and biotin were omitted, CaCl_2 was increased to 5.2 mM, and 0.15% yeast extract was included). Transition through the cell cycle was monitored by smearing small fragments in 50% glycerol-ethanol and examining by phase contrast microscopy. $^{45}\text{Ca}^{2+}$ (final concentration $2\ \mu\text{Ci ml}^{-1}$) was added (at 'zero' time) to the growth medium of a macroplasmodium either during interphase before the third mitosis (a) or shortly before it entered the third mitosis (b). $^{45}\text{Ca}^{2+}$ uptake was measured by excising small pieces of plasmodium at intervals after $^{45}\text{Ca}^{2+}$ addition, washing twice by immersion for 10 s in medium containing a 10-fold excess of unlabelled Ca^{2+} , then rinsing briefly in distilled water to remove traces of the medium. The plasmodium was scraped from the filter paper and homogenised in 1 ml of distilled water. Aliquots were taken for liquid scintillation counting and protein assay⁸. The scales are different for the ordinate of the two traces as ^{45}Ca accumulation fluctuates more intensively during interphase. P designates prophase; M, metaphase; and A anaphase as observed by microscopy⁹. The inset shows a tracing of the pattern of A_{380} changes observed in a Varian 635 spectrophotometer when light is directed through a small region of plasmodium growing on an agar-coated piece of Perspex, following the method of Sachsenmaier *et al.*². The maximum absorbance change in peaks represents 0.05 units.

anaphase, a large overall accumulation of $^{45}\text{Ca}^{2+}$ once again takes place.

The correlation between Ca^{2+} uptake and mitotic phases is clearer when 5-min pulses with ^{45}Ca are monitored. The results of three such experiments are shown in Fig. 2 and similar patterns were obtained in seven other experiments. Such reproducible cycles in Ca^{2+} uptake were not as evident when the pulse time was shortened to 3 or lengthened to 7.5 min. Peaks in Ca^{2+} uptake are found to coincide with prophase and anaphase while uptake is low during metaphase. The time course of Ca^{2+} uptake during metaphase (Fig. 2 inset) indicates that in the 5-min pulse period, $^{45}\text{Ca}^{2+}$ is initially taken up but is then released back into the medium; in prophase and anaphase, by contrast, uptake is continuous and approximately linear with time. Uptake of Ca^{2+} during interphase is more irregular as the initial 5-min period in Fig. 1 indicates.

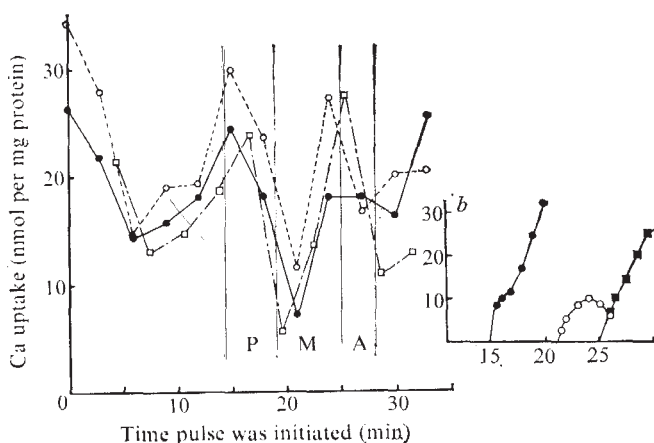
The large amplitude cycles of $^{45}\text{Ca}^{2+}$ uptake are not reflected in fluctuations in the total amount of Ca^{2+} present in the plasmodia. Samples taken during mitosis gave a range of values from 233 to 254 nmol Ca^{2+} per mg protein when measured by atomic absorption spectroscopy, showing no correlation with the pulse experiments. These results show that the cyclical uptake of Ca^{2+} during mitosis may be into particular pool(s)

within the plasmodium, rather than being due to generalised uptake and release of a large fraction of cellular Ca^{2+} and that this pool is not in equilibrium with the major pool of Ca^{2+} present in the plasmodium. Although mitochondria¹¹, cytoplasmic vesicles¹², and endoplasmic reticulum¹³ have been identified as sites for Ca^{2+} sequestration in *Physarum*, we have not so far been able to identify a specific 'mitotic' pool of Ca^{2+} in nuclei, mitochondria or other particulate fractions of the protoplasm.

We have found that Ca^{2+} influx in microplasmodia is largely energy dependent, while efflux is stimulated by KCN. A similar response is observed in the yeast *Schizosaccharomyces pombe*¹⁴. To determine whether the fluctuations observed in Ca^{2+} uptake during mitosis are related to variations in respiratory energy supply, oxygen uptake rates during mitosis were examined. The pattern observed (Fig. 3) is almost identical to that for Ca^{2+} uptake. If the 5-min pulse period for Ca^{2+} uptake is taken into account, the coincidence of the curves could indicate that the changes in O_2 uptake precede those in Ca^{2+} uptake. Whether this relationship is a direct consequence of mitochondrial Ca^{2+} uptake or is related to respiratory energy production is not certain although the effect of caffeine on the motility of plasmodial fragments¹⁵ suggests that a caffeine-sensitive reticulum system is involved in protoplasmic motility. Energy-dependent uptake of Ca^{2+} could involve an active transport system in the plasma membrane, energy-linked endocytosis, or Ca^{2+} sequestration by intracellular organelles, all of which may depend on respiratory energy.

How might the movement of Ca^{2+} into and out of *Physarum* plasmodia be related to the events of mitosis? In heart and smooth muscle cells, an influx of external Ca^{2+} is believed to trigger a release of Ca^{2+} from intracellular stores¹⁶. In sea-urchin eggs, a vesicular system associated with the mitotic apparatus may regulate the local concentration of Ca^{2+} ions¹⁷. It is thus possible that the prophase and anaphase peaks of Ca^{2+} uptake we observe trigger Ca^{2+} release in or near nuclei, thus initiating or in some other way regulating chromosomal movement. During metaphase, when microtubules proliferate and chromosomal movements temporarily cease, a lower rate of influx of

Fig. 2 Uptake of $^{45}\text{Ca}^{2+}$ during mitosis in *P. polycephalum*. During the third synchronous mitosis (24–26 h after initiation), sectors of plasmodium on filter paper (approximately $1 \times 2\text{ cm}$) were removed and placed on a 200- μl drop of medium containing $^{45}\text{Ca}^{2+}$ ($2\ \mu\text{Ci ml}^{-1}$) at the times indicated. After incubation for 5 min at 25 °C the sector was treated as described in Fig. 1 and assayed for radioactivity and protein. a, $^{45}\text{Ca}^{2+}$ uptake observed in three separate experiments (■, ●, ○) during mitosis. b, Time course of $^{45}\text{Ca}^{2+}$ uptake over the 5-min pulse period during prophase (●), metaphase (○), and anaphase (■). Uptake over 5 min during interphase is shown in Fig. 1a. The time course of uptake was reproducible for each phase of the mitotic cycle in three experiments, although data is given here for a single experiment. The zero points for calcium uptake in (b) represent the times when the segments were placed on labelled medium.



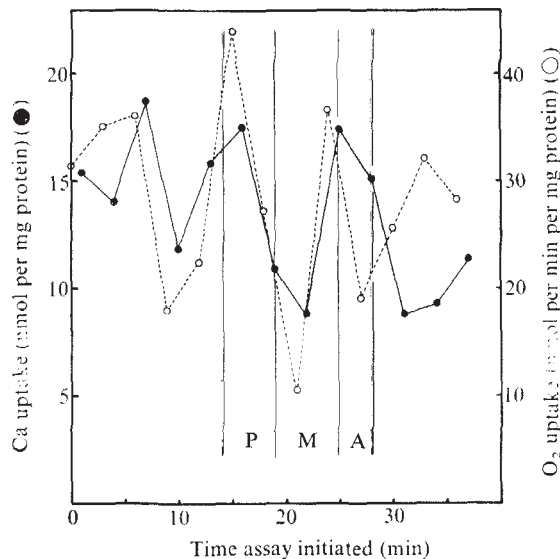


Fig. 3 Comparison of oxygen and $^{45}\text{Ca}^{2+}$ uptake during mitosis. Segments of plasmodium (approximately 1 cm^2) were excised from a macroplasmodium during mitosis and the plasmodial segment was removed from its filter paper support by immersing in growth medium and gently prising it free. Oxygen uptake ($\circ$) was measured in a Gilson oxygraph with a Clark-type oxygen electrode by placing the plasmodial piece in 3 ml of respiration buffer containing 350 mM mannitol and 10 mM MES buffer at pH 6.0. Each point represents the initial O_2 uptake rate at the time when the segment was placed in the oxygen electrode. Rates were usually linear for at least 1 min. Ca^{2+} uptake ($\bullet$) was measured in the same plasmodium 1 min later using the 5-min pulse method described in Fig. 2.

Ca^{2+} may be necessary to reduce local Ca^{2+} concentrations so that tubulin polymerisation is promoted³.

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Killed *Listeria monocytogenes* vaccine becomes protective on addition of polyanions

In the interaction between macrophages and intracellular parasites, microbial components which prevent phagosome-lysosome fusion^{1,2} have a decisive role. The resistance to facultative intracellular parasite *Listeria monocytogenes* is a form of cell-mediated immunity³, as it can be passively transferred with viable lymphoid cells⁴. Resistance to *listeria* infection can be induced by sublethal numbers of

Table 1 Resistance to infection following treatment with DS 500 and immunisation with dead *listeria*

No. of bacteria	DS 500 dose (mg per kg body weight)				
	50	25	12.5	5	None
10^9	3/4*	6/6	5/6	4/6	0/6
10^8	6/6	6/6	0/6	0/6	0/6
10^7	0/6	0/6	0/6	0/6	0/6
None (saline)	0/6	0/6	0/6	0/6	0/6

Groups of BALB/c mice were injected i.p. with DS 500 or saline 1d before i.p. injection of heat-killed (56°C for 60 min) *listeria* or saline. Seven days later all animals were challenged i.p. with 50 LD_{50} *listeria*. The numbers of survivors on the numbers of mice challenged were recorded 2 weeks after challenge.

*In this group two mice died after immunisation.

viable *listeria*^{5,6}. Induction of resistance by non-viable *listeria*, however, is effective only after repeated injections of *listeria* preparations in combination with lipopolysaccharides⁷. Lipopolysaccharide is cytotoxic against macrophages^{8,9}, suggesting that the difference in processing of viable and dead microorganisms by macrophages might explain the induction of resistance by vaccine of live and not of dead bacteria. Impairing macrophage activity might result in a processing of dead *listeria* advantageous for the induction of resistance. We show here that killed *L. monocytogenes* vaccine becomes protective when the polyanions dextran sulphate (DS 500, molecular weight 500,000, Serva) and suramin (Bayer) are added. These polyanions are known to inhibit phagosome-lysosome fusion in macrophages^{2,10}. Moreover, dextran sulphate is a potent adjuvant for both humoral¹¹ and cell-mediated responses¹². The latter might be particularly important in view of the requirement of cell-mediated immunity for resistance to *listeria*³.

BALB/c mice were injected intraperitoneally (i.p.) with graded doses of DS 500 or suramin and 1 d later with different numbers of heat-killed *listeria*. The challenge injection of $50 \times \text{LD}_{50}$ (dose causing 50% killing) was given i.p. 7 d later. Pretreatment with DS 500 allowed induction of resistance with 10^9 and 10^8 dead *listeria* (Table 1). With the latter numbers of bacteria protection could only be induced following high doses of DS 500. The dose of 50 mg DS 500 per kg body weight probably damaged the phagocytic system to such a degree that 10^9 *listeria* were badly tolerated as an immunising dose.

Doses of DS 500 over 50 mg per kg in combination with 10^7 *listeria* did not result in protection. The protection afforded by suramin treatment was slightly less (Table 2). Complete protection was only found in the combination of highest doses suramin and *listeria*. But again one animal did not survive the immunisation.

The doses of suramin required to give protection in addition to injections of dead *listeria* are about 10 times as high

Table 2 Resistance to infection after treatment with suramin and immunisation with dead *listeria*

No. of bacteria	Suramin dose (mg per kg body weight)					
	500	300	150	50	15	None
10^9	5/5*	5/6	5/6	4/5	4/5	0/3
10^8	4/6	2/6	1/6	0/4	0/4	0/3
10^7	0/6	0/6	0/6	ND	ND	ND
None (saline)	0/6	0/6	0/6	0/6	0/3	0/3

Experimental details as for Table 1. ND, Not done.

*In this group one mouse died after immunisation.

as those of DS 500. A similar ratio of activities was found for *in vitro* inhibition of phagosome-lysosome fusion where the optimal inhibiting doses of suramin and DS 500 were respectively 200 and 30 µg (M. J. de Reuver, personal communication). The influence of the interval between DS 500 and immunising injection was studied using 30 mg DS 500 per kg (Table 3). With an interval of 8 h between DS 500 and 10⁹ listeria all mice died after immunisation (data not shown). Reduction of the dose of DS 500 to 15 mg per kg, followed by 10⁸ bacteria resulted in complete protection (Table 3). This suggests that the interval between injections of the toxic products DS 500 and dead listeria should not be too small if high doses of both are used.

Host immunity and survival of intracellular parasites depend on a delicate balance of host-parasite interplay. The ingestion of previously killed intracellular parasites by macrophages leads to rapid and extensive phagosome-lysosome fusion¹³, but no resistance to infection. Resistance to intracellular growing bacteria can in general only be achieved by immunisation with viable bacteria. Impairing macrophage function by DS 500 resulted in increased susceptibility to infection with listeria^{14,15}. These results suggest that although macrophages are host cells for listeria,

Table 3 Effect on resistance to infection of the interval between DS 500 treatment and immunisation with dead listeria

No. of bacteria	DS 500 treatment on				No treatment
	Day -2	Day -1	-8 h	Day +1	
10 ⁹	5/5*	6/6	6/6	5/5*	0/6
10 ⁸	3/6†	6/6	2/6	0/6	0/6
None (saline)	0/6	0/6	0/6	0/6	0/6

Groups of BALB/c mice were injected i.p. with 30 mg DS 500 per kg before or after injection of dead listeria or saline (controls). The groups receiving injections with the 8 h interval were treated with 15 mg DS 500 per kg. Other details as for Table 1.

*In these groups one mouse died after immunisation.

†One mouse died within the 2 weeks recording period after the challenge, but two mice died 1 week later.

the degree of processing of the listeria is decisive for the induction of resistance. The probably incomplete processing of viable listeria by macrophages seems to be a prerequisite for the induction of resistance. The more complete digestion of non-viable listeria does not result in protection.

The finding that lower numbers of non-viable bacteria (10⁶) needed high doses of polyanions to obtain protection supports the hypothesis that the degree of digestion of bacteria should be limited in order to induce resistance. Experiments are in progress to isolate the fraction(s) of listeria responsible for the induction of resistance.

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Non-infectious virus induces cytotoxic T lymphocytes and binds to target cells to permit their lysis

MUCH current interest is focused on the involvement of expressed products of the major histocompatibility (*H*-2) gene complex, especially those associated with the *K* and *D* regions of *H*-2, in inducing cell-mediated immune responses and in permitting recognition and killing by cytotoxic thymus-derived lymphocytes (CTL). Observations in many laboratories have led to the generalisation that syngeny between stimulator and target cells at the *K* and/or *D* region of *H*-2 is required for CTL activity to virus-infected cells¹⁻³, minor histocompatibility antigens⁴, the male *H*-Y antigen⁵ and chemically modified syngeneic cells⁶⁻⁹. This study asks three questions related to the genetic restriction of induction and expression of CTL: (1) can non-infectious virus efficiently induce CTL, (2) can non-infectious virus absorbed to target cells render them susceptible to killing by CTL, and (3) can non-infectious virus absorbed onto or chemically linked to cells induce CTL and does using allogeneic or xenogeneic carrier cells affect this induction?

Two major models have been proposed to explain the involvement of *H*-2 antigens and foreign antigens, such as viral antigens, in recognition of target cells by CTL. The 'dual recognition' model proposes recognition of *H*-2 and foreign antigens on the target cell surface by two separate receptors on the CTL while the 'adaptor-antigen complex' or 'modified self' model postulates a physical association between *H*-2 antigens and the foreign antigen on the cell surface to form hybrid antigens containing elements of self and non-self^{10,11}.

Using 6/94 virus, a para-influenza type 1 virus antigenically related to Sendai virus, it has been shown that although only virus-infected cells histocompatible with the effector T cells can serve as susceptible target cells, syngeneic, allogeneic, and even xenogeneic virus-infected cells are capable of inducing virus-specific cytotoxicity¹². But, as the virus used in the previous study retained some infectivity, it was possible that the allogeneic and xenogeneic virus-infected cells used to induce CTL released

Table 1 Characterisation of anti-6/94 effector cells

Treatment	Live 6/94	BPL 6/94
Hold on ice	58	39
4 h at 37 °C	32	31
Nylon column nonadherent	58	48
Guinea pig serum (C)	54	39
Anti-Thy 1.2+C	4	6
Rabbit α mouse Ig+C	ND	70
Rabbit α 6/94+C	ND	34

BALB/c mice were injected i.p. with 100 µg of live or BPL-treated 6/94 virus. On day 5, spleen cell suspensions were prepared, treated as indicated and assayed against P815Y target cells incubated for 90 min with BPL-treated 6/94 virus immediately before labelling with Na₂⁵¹CrO₄. Cytotoxicity values are % specific lysis for an 18-h assay and spleen cell: target cell ratio of 50:1. Spontaneous release was 47%. Treatment with anti-Thy 1.2 serum removed 38% of the cells. ND, Not determined.

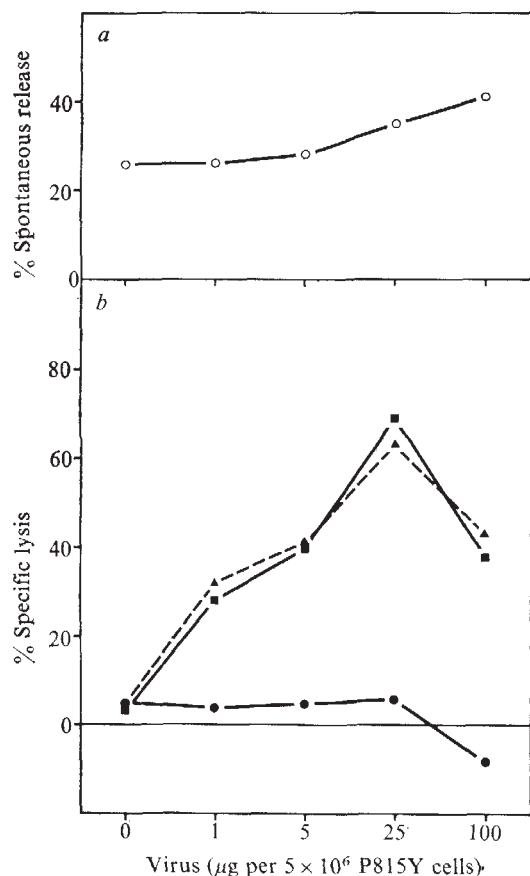


Fig. 1 Induction of anti-viral cytotoxic thymic-derived lymphocytes (CTL) by infectious and non-infectious 6/94 virus with assay on target cells pre-incubated with different amounts of non-infectious 6/94 virus. BALB/c ($H-2^d$) mice were injected i.p. with 100 μg of live 6/94 virus in phosphate buffered saline (▲), with 100 μg of non-infectious 6/94 virus treated with BPL¹⁸ and verified negative for infectivity by serial egg passage and incubation with susceptible cells in culture (■), or uninjected (●). On day 5 spleens were removed and assays were performed for 18 h against P815Y ($H-2^d$) cells incubated with 0, 1, 5, 25 or 100 μg BPL-treated 6/94 virus (5×10^6 cells in 2 ml, 37°C, 90 min, washed and then labelled with ^{51}Cr chromate). All assays were carried out using lymphocyte-target cell ratios of 200:1, 67:1, and 22:1. Only data for 67:1 are shown for simplicity. *a*, Shows spontaneous release in 18 h as a % of total incorporation. *b*, Cytotoxicity in terms of % specific lysis.

virus which then infected host cells; the measured cytotoxicity might then have been induced by virus-infected (syngeneic) host cells.

To compare the immunogenicity of live and non-infectious 6/94 virus, BALB/c mice ($H-2^d$) were injected intraperitoneally (i.p.) with 100 μg of either infectious or β -propiolactone (BPL) inactivated¹⁷ 6/94 virus. Lymphocytes from these mice and uninjected controls were then assayed on day 5 against P815Y cells ($H-2^d$) incubated with 0, 1, 5, 25 or 100 μg of BPL inactivated 6/94 virus for 90 min before labelling with radioactive chromate. Figure 1b demonstrates that live and non-infectious 6/94 virus induced the same amount of cytotoxicity and that P815Y cells were made susceptible to killing when incubated with as little as 1 μg of non-infectious virus just before labelling. The rate of spontaneous release is increased as more virus is absorbed to the target cells (Fig. 1a), indicating that optimum assay conditions are a compromise between more virus per cell and increasing spontaneous release.

The cytotoxicity induced by both live and non-infectious 6/94 virus was shown to be mediated by CTL (Table 1). The cytotoxicity induced by both live and non-infectious 6/94 virus was not labile at 37°C as is true for 'natural'

killer cells^{13,14}. The activity was largely abolished by anti-Thy 1.2 serum and complement and was not diminished by removal of cells adherent to nylon wool or of cells killed by anti-mouse immunoglobulin and complement; it seems therefore that lysis is mediated by a T cell and not by a B cell or macrophage.

Non-infectious 6/94 virus was absorbed on to (1) P815Y cells which are $H-2^d$ like BALB/c; (2) EL4 cells which are $H-2^b$ and allogeneic to BALB/c, and (3) bovine kidney cells (MDBK) which are xenogeneic to BALB/c; all cells were washed extensively to remove any free virus, and injected i.p. into BALB/c mice.

Table 2 shows that 6/94 virus absorbed to all three cell types induced cytotoxicity against P815Y cells sensitised with either infectious or BPL-treated 6/94 virus. The 6/94 virus, like the well characterised Sendai virus, adsorbs rapidly to the cell surface, fusing its envelope with the cell membrane as a normal event in penetration¹⁵; once this occurs, therefore, virus would not be expected to desorb from the cells used for the injection of mice. To make it even less likely that the effective immunogen is free virus or virus that desorbed from the injected cells and reabsorbed to host cells, the xenogeneic MDBK cell and 6/94 virus were covalently linked using four additions at 10 min intervals of freshly diluted *bis*-diazotized benzidine (BDB) solution¹⁶. The results in Table 2 show that 6/94 virus covalently linked to xenogeneic cells is still able to induce cytotoxicity against 6/94 determinants on P815Y cells.

As was shown in work with CTL induced with 6/94 virus¹², we did not observe significant cell-mediated cytotoxicity of allogeneic EL4 cells or xenogeneic bovine kidney cells adsorbed with 6/94 virus. But, the lack of cytotoxicity by CTL on allogeneic and xenogeneic targets was not due to lack of expression of viral determinants or to an inability of the cells to be lysed, because all these cells were lysed by rabbit anti-6/94 virus antiserum and guinea pig complement (Table 3). This was also true for MDBK cells to which virus had been bound covalently. The P815Y and EL4 cells did not survive the BDB coupling conditions and hence were not tested.

In summary, our results show that non-infectious virus is immunogenic both as free virus and when fused with cells of the same or different $H-2$ as the host. Non-infectious virus covalently linked to xenogeneic cells also was immunogenic. These results make less likely, but *in vivo* experiments can never completely exclude, the possibility that macrophages process free or cell-bound virus and present viral determinants on a host $H-2$ background. Immunogenic determinants in these experiments are contained in the 6/94 virus and do not involve a complex with structures present on the cell carrying the virus. This finding and

Table 2 Induction of CTL with non-infectious 6/94 virus passively adsorbed to or covalently linked with *bis*-diazobenzidine to various cells

Immunising cells	P815Y	Target cells P815Y (BPL 6/94)	P815Y (live 6/94)
None	1	6	9
P815Y (BPL 6/94)	8	39	47
EL4 (BPL 6/94)	5	35	26
MDBK (BPL 6/94)	—7	75	55
MDBK (BDB coupled BPL 6/94)	6	55	51

BALB/c mice were immunised with the indicated cells and assayed on day 5 for 18 h at 150:1 spleen cells: target cell against P815Y incubated with live or BPL-treated 6/94 virus or left without virus. Values are % specific lysis for the mean of triplicate determinations. Standard error of the mean was always less than 2 percentage points or 10% of the mean. Spontaneous release was 31% for P815Y, 54% for P815Y (BPL 6/94) and 48% for P815Y (live 6/94).

Table 3 Lysis of various target cells with anti-6/94 antibody and complement

	Uninfected	+ Live 6/94	+ BPL 6/94	Coupled BPL 6/94
P815Y	25	52	70	—
EL4	2	69	75	—
MDBK	2	42	34	47

For each target, 2×10^5 ^{51}Cr labelled cells in 50 μl were mixed with 50 μl 1:4 or 1:20 dilutions of rabbit anti-6/94 antibody, 50 μl of a 1:9 dilution of guinea pig complement was added. The mixture was incubated for 1 h at 37 °C, and one half the supernatant was counted. Values are % specific lysis for 1:20 initial dilutions of antiserum. Spontaneous release ranged from 3% to 24% for the ten different targets.

the restriction of lysis by CTL of histocompatible target cells displaying virus argues for a view of CTL possessing separate receptors for H-2 and for virus determinants. Thus, while it seems that both receptors must be bound for CTL activity to be expressed, CTL activity is apparently induced by viral determinants alone without involvement of histocompatibility antigens.

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Effects of a nonapeptide FTS on lymphocyte differentiations *in vitro*

BACH *et al.*^{1,2} have reported the amino acid sequence of a putative thymic hormone isolated from pig serum³, a nonapeptide called FTS (*facteur thymique serique*). Natural and synthetic FTS showed high activity in the rosette test used as a bioassay^{1,2}. Bioassays for thymic hormones⁴, however, can be spuriously triggered by many substances of non-thymic origin⁵, a finding probably related to mediation by a cyclic AMP second signal⁶. Early steps in B-cell differentiation are also mediated by cyclic AMP (refs 6–9) and comparison of induction of T cells and B cells from committed precursor cells *in vitro* (the dual induction assay) enables a distinction to be made between selective and non-selective inducing agents^{7,8}. This is exemplified by induction studies in the mouse with

thymopoietin^{10,11} and ubiquitin^{7,12,13}. Bovine thymopoietin selectively induces T-cell differentiation and actually inhibits B-cell differentiation⁹. By contrast, ubiquitin is non-selective, inducing both T- and B-cell differentiation⁷. Ubiquitin at high concentrations does not induce differentiation whereas these high concentrations do not impair induction by thymopoietin or other agents^{8,14}. Another feature of induction by ubiquitin is its inhibition by the β -adrenoceptor-blocking drug propranolol, which is without effect on induction by thymopoietin⁷. We have evaluated synthetic FTS in the dual induction system in the chicken to determine whether it showed inductive selectivity appropriate to a thymic hormone, and have found that it induced non-selective differentiation of both T cells and B cells and closely resembled ubiquitin in its activity.

The nonapeptide, $\text{NH}_2\text{-Gln-Ala-Lys-Ser-Gln-Gly-Gly-Ser-Asn-COOH}$, was synthesised using solid-phase methodology (Peninsula, San Carlos, California). The purified peptide showed a single spot in four thin-layer chromatography systems and on high voltage electrophoresis at pH 6.5. After acid hydrolysis, the amino acid composition showed appropriate molar ratios. Ubiquitin was isolated as described previously⁷. The dual induction assay for T-cell and B-cell differentiation in fractionated bone marrow cells from newly hatched chickens was carried out as described previously⁸. Synthetic FTS induced both T-cell and B-cell differentiation (Fig. 1) with high dose inhibition at the higher concentrations ($100 \mu\text{g ml}^{-1}$). B-cell induction was only obtained consistently at a concentration of $1 \mu\text{g ml}^{-1}$, whereas T-cell induction was obtained over the broader concentration range of 1 ng ml^{-1} to $1 \mu\text{g ml}^{-1}$. More detailed information about the mechanism of induction by FTS was obtained from inhibition studies, the inhibiting agent being added at the same time as the inducing agent (Table 1). Inductive concentrations of FTS ($1 \mu\text{g ml}^{-1}$) were inhibited by inhibitory concentrations of ubiquitin ($100 \mu\text{g ml}^{-1}$), and inductive concentrations of ubiquitin ($0.5 \mu\text{g ml}^{-1}$) were inhibited by inhibitory concentrations of FTS ($100 \mu\text{g ml}^{-1}$). Inductive concentrations of both FTS and ubiquitin were inhibited by propranolol 10^{-5} M , which did not affect induction by cyclic AMP.

Fig. 1 Relationship between concentration of synthetic FTS and induction of Bu-1⁺ (a) or Th-1⁺ cells (b) in fractionated bone marrow from newly hatched chickens. Bone marrow cells from newly hatched chickens were fractionated by ultracentrifugation on a discontinuous bovine serum albumin gradient, and cells from the lighter layers were incubated with or without various concentrations of FTS. After incubation for 2.5 h, cells were washed and tested in the cytotoxicity assay using anti-Bu-1 and anti-Th-1 alloantisera and a mixed avian-mammalian complement system as described previously⁸. FTS induced both Bu-1⁺ and Th-1⁺ cells and showed high-dose inhibition (six experiments, each line represents one experiment).

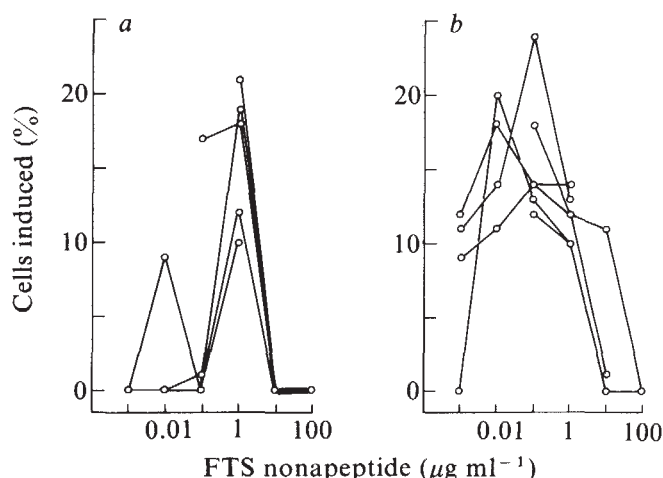


Table 1 Inhibitor studies with synthetic FTS and ubiquitin in the dual induction assay using chicken cells

Inducing agent	Inhibiting agent	Cells induced (%)	
		B cells (Bu-1 ⁺)	T cells (Th-1 ⁺)
FTS 1 $\mu\text{g ml}^{-1}$	—	11,16	11,19
FTS 1 $\mu\text{g ml}^{-1}$	Ubiquitin 100 $\mu\text{g ml}^{-1}$	1,0	2,0
—	Ubiquitin 100 $\mu\text{g ml}^{-1}$	1,0	0,0
Ubiquitin 0.5 $\mu\text{g ml}^{-1}$	—	9,16	20,16
Ubiquitin 0.5 $\mu\text{g ml}^{-1}$	FTS 100 $\mu\text{g ml}^{-1}$	0,0	0,0
—	FTS 100 $\mu\text{g ml}^{-1}$	0,0	0,0
FTS 1 $\mu\text{g ml}^{-1}$	—	15,22	18,20
FTS 1 $\mu\text{g ml}^{-1}$	Propranolol 10 ⁻⁵ M	0,0	0,0
Ubiquitin 0.5 $\mu\text{g ml}^{-1}$	—	19,11	15,17
Ubiquitin 0.5 $\mu\text{g ml}^{-1}$	Propranolol 10 ⁻⁵ M	0,0	0,0
Cyclic AMP 0.2 $\times 10^{-3}$ M	—	18,8	21,17
Cyclic AMP 0.2 $\times 10^{-3}$ M	Propranolol 10 ⁻⁵ M	19,13	11,14

High concentrations of FTS or ubiquitin inhibited inductive concentrations of the other. Inductive concentrations of either were inhibited by propranolol.

Each figure represents the mean of duplicate or triplicate tubes in a single experiment.

The amino acid sequence of FTS (refs 1, 2) differs from that of thymopoietin^{10,11}, and the biological activity of these two peptides in the dual induction assay was also quite different. Bovine thymopoietin was inactive in the dual induction assay in the chicken⁶. Unlike bovine thymopoietin⁷, porcine FTS was both active and non-selective in the dual induction assay using chicken cells and was inhibited by propranolol. By contrast, our findings show a close similarity between the activity of FTS and that of ubiquitin, in that both caused non-selective induction in the dual induction assay; induction by both was inhibited by the β -adrenoceptor-blocking agent, propranolol; and in that high concentrations of each inhibited induction by inductive concentrations of the other. This finding is especially noteworthy because the primary structure of FTS does not closely resemble that of ubiquitin^{12,13}; the similarity of tertiary structure of the active sites implied by the similarities of activity of these two peptides must be generated by folding of chains of dissimilar primary amino acid sequence. Evolutionary convergence rather than modification of a common ancestral gene would be the likely cause of this relationship¹⁴.

We conclude that FTS is a biologically active molecule that induces lymphocyte differentiation, and that the biological activity of FTS mimics ubiquitin, a polypeptide which induces lymphocyte differentiation non-selectively by reason of having an adrenomimetic active site. Our observations indicate that FTS is probably not a thymic hormone, but they do confirm that this circulating nonapeptide can induce lymphocyte differentiation. These findings do not detract from interest in this factor. Rather, they raise important questions concerning its origin in the body and a possible physiological role in regulating lymphocyte differentiation and maturation.

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Expression of MuLV GP71-like antigen in normal mouse spleen cells induced by antigenic stimulation

ALL mouse strains investigated possess cellular DNA sequences which are homologous to the genomes of endogenous C-type particles¹. Also, major envelope glycoproteins of the oncoviruses², such as GP71, are found on the surface of normal cells and they display a considerable polymorphism. An antigen serologically indistinguishable from AKR murine leukaemia virus (MuLV) GP71 is present in normal bone marrow cells of all mouse strains investigated but not in normal spleen cells³. Allogeneic⁴ and mitogenic stimulation^{5,6} of spleen cells have been reported to result in C-type particle synthesis in some but not all mouse strains. We report here that both T and B lymphocytes of mice express a GP71-like antigen on their surfaces if antigenically stimulated.

Goat⁷ or rabbit⁸ antisera prepared against isolated GP71 of Friend leukaemia virus (FLV) were applied to the mouse lymphocytes. In some experiments, a goat antiserum prepared against dissociated feline leukaemia viruses (FeLV) was included⁹. These antisera possess antibodies to type-, group- and interspecies-specific determinants of MuLV GP71 (ref. 10) and thus can react with the major glycoprotein moieties of all C-type murine oncoviruses. The antisera and the normal control sera were thoroughly absorbed with fresh suspension of liver and spleen cells of the mouse strains used in a particular experiment. Fresh rabbit sera served as source of complement. Mishell-Dutton-type cultures of mouse spleen cells activated by sheep red blood cells (SRBC) were used to examine the effect of anti-GP71 antisera on the primary immune response *in vitro*¹¹. The immune responses were assessed by the haemolytic plaque test of Jerne¹² as modified by Mishell and Dutton¹¹. The number of plaque-forming cells (PFC) routinely increases after 3 d of culture and usually reaches an optimum on day 5.

Table 1 shows that the addition of anti-GP71 antiserum to spleen cultures of F₁ hybrid mice (C57B1/6 $\times$ DBA/2 = B₆D₂) on day 3 of culture significantly reduced the number of PFC

discovered on day 5 dependent on the presence of active complement. Since the anti-SRBC response is T-cell dependent¹³, either T or B lymphocytes or both may be affected by the treatment. To determine whether or not antibody-producing B cells can thus be affected, cultured cells were treated directly before the plaque assay when all of the antibody-producing B cells were already present.

While the controls treated with normal goat serum (NGS) and complement (C) contained 4,147 PFC per 10^6 recovered cells, treatment with anti-FLV GP71 antiserum and C resulted in a drastic reduction (125 PFC per 10^6 cells = 3%). Plaque-forming cells were similarly reduced by treatment with anti-FeLV antiserum and complement (65 PFC per 10^6 cells = 1.6%). This suggests that the induced antigen is in fact viral GP71 and not simply another membrane structure displaying a chance cross-reactivity. Anti FLV GP71 antiserum reacts predominantly through group-specific determinants on the GP71 molecules of all mouse oncoviruses while anti-FeLV antiserum can only detect their interspecies-specific determinants. Thus, antibody-producing B lymphocytes of $B_6D_2F_1$ mice express sufficient GP71-like antigens on their surface to be eliminated by corresponding antisera and complement.

Examination of a plaque centre cell revealed actual C-type particle synthesis by these antibody-producing cells. Immunological induction of GP71-like viral antigens is, however, also possible in lymphocytes of NIH-Swiss mice. These mice only possess a xenotropic provirus¹⁴.

The following experiments were designed to find out whether or not T-helper cells also express oncoviral antigens on immunological stimulation. For this purpose, C57B1/6 mice were primed and boosted with dinitrophenyl (DNP) KLH *in vitro* using the method of North and Askonas¹⁵. In secondary spleen cultures derived from those mice and restimulated with DNP-KLH, a considerable IgG immune response is obtained (Table 2). The removal of T-helper cells by treatment with anti-Thy 1 and complement before the culture prevents the immune response which, however, can be reconstituted by addition of specifically antigen-activated T cells¹⁶ (Table 2). These reconstituting T-helper cells, however, are sensitive to anti-FLV GP71 antiserum and complement, indicating that, like immunologically activated B lymphocytes, they also express the GP71-like antigen. This is of particular interest since concanavalin A stimulation of C57B1/6 spleen cells leads to the induced expression of GP71-like antigens without C-type particles synthesis in contrast to DBA/2J or $B_6D_2F_1$ cultures where both occurs (manuscript in preparation).

Our results suggest that both T and B lymphocytes of mice, if antigenically stimulated, either *in vivo* or *in vitro*, display GP71-like antigens on their surfaces where they are accessible to antibodies.

To demonstrate more directly that unstimulated lymphocytes do not express these antigens, the following experiment was performed. Spleen cells of mice primed and boosted with DNP KLH were treated with normal goat or with goat anti-FLV GP71 antisera and complement before culture. One set of cultures

Table 2 Effect of anti-FLV GP71 antiserum and complement on primed spleen cells of C57B1/6 mice

Treatment before culture	PFC per 10^6 cells (anti-DNP IgG) day 5	% Control
None (control)	4,716	100
T-helpers reduced (TR)	245	5
TR + untreated helpers	4,820	102
TR + treated helpers (NGS + C')	2,498	53
TR + treated helpers (GP71 + C')	789	16

Mice were primed i.p. with 200 μ g of dinitrophenyl (DNP) KLH (312 DNP groups per 10^6 molecular weight) and 10^9 *Bordetella pertussis* organisms. The mice were boosted with 20 μ g DNP KLH i.p. 7 d before killing. Spleen cells were cultured directly with DNP-KLH or after treatment with mouse anti-Thy 1 and complement¹⁶. T-helper cells were obtained from C57B1/6 mice primed with KLH and boosted 3 d before use. In reconstitution experiments, 1×10^6 Thy 1 + C'-treated cells were cultured with 4×10^6 helper cells in 0.5 ml of medium. Treatment of helper cells with anti-FLV GP71: 3×10^7 cells, 30 min on ice with 0.5 ml of 1:5 diluted serum. After washing, cells were incubated with 0.5 ml of 1:5 diluted rabbit complement and washed twice.

received DNP-KLH, another SRBC. The secondary immune response to DNP-KLH *in vitro* was practically abrogated by the antiserum treatment (Fig. 1). Although the anti-SRBC response was also reduced, it still was about 100-times higher on a percentage of control basis than the anti-DNP response. This suggests a remarkable specificity of the treatment which indeed seems to predominantly effect antigen-activated lymphocytes (DNP-specific) while unstimulated cell clones (SRBC-specific) are preserved. In principle, an antigen-specific immune suppression seems to be possible in these experimental conditions by applying appropriate antisera against oncoviral antigens such as GP71. Also, using type-specific antisera, it will now be possible to characterise the type of endogenous viruses, the antigens of which

Fig. 1 Specificity of anti-GP71 antisera for antigen-activated lymphocytes. DBA/2 mice were used. The condition of *in vivo* priming and boosting, and antiserum treatment before culture were as described in Table 2. One set of cultures received KLH-activated helper cells and DNP-KLH as antigen, the other received SRBC and TRF on day 2 for reconstitution of T-helper function¹⁷. Samples a-d all underwent T-cell reduction. a, NGS + C' + helper; b, anti-GP71 + C' + helper; c, NGS + C' + helper; d, anti-GP71 + C' + helper. The value for b is 0.3%.

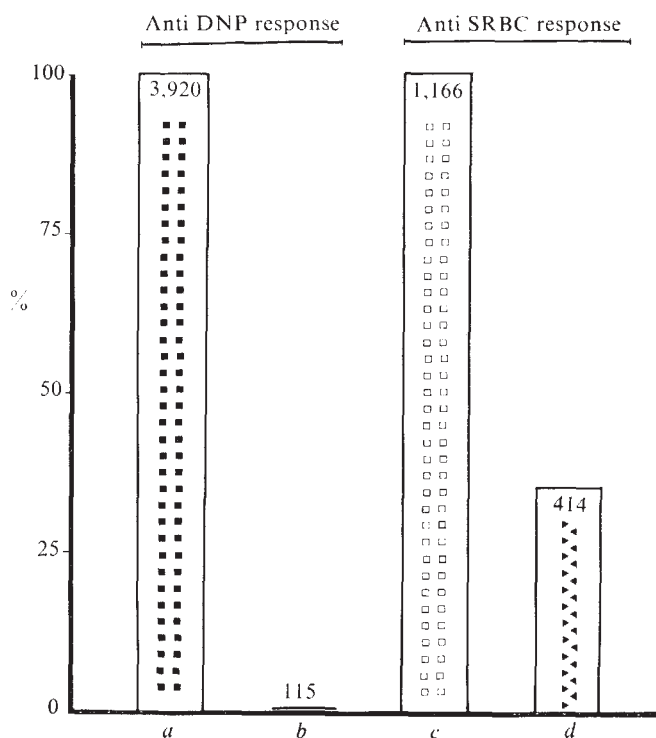


Table 1 Effect of anti-GP71 antiserum and complement on anti-SRBC response *in vitro*

Additions on day 3	PFC per 10^6 cells, day 5 (anti-SRBC IgM)	% Control
None (control)	1,377	100
NGS + complement	1,033	75
Anti GP71 + complement	197	14
Anti GP71 + inactivated complement	1,522	110
Inactivated anti GP71	1,640	119
Inactivated anti GP71 + complement	88	6

5×10^6 spleen cells of $B_6D_2F_1$ mice in 0.5 ml of medium containing 5% FCS were cultured together with 2.5×10^6 SRBC¹¹. On day 3, cultures received 0.1 ml of the respective sera diluted 1:5 and 0.05 ml of undiluted rabbit complement or 0.05 ml of medium. 1.5 ml of the diluted sera had been absorbed for 3 h on ice with suspended cells equivalent to one liver and one spleen of $B_6D_2F_1$ mice. Heat inactivation was performed 30 min incubation at 60 °C.

can be expressed on lymphocyte surfaces of the various mouse strains.

These findings are compatible with the notion that the expression of endogenous viral antigens may generally have a role in cellular proliferation and/or differentiation. Using cells of the immune system might prove quite helpful in studying these questions. All immune reactions are characterised by proliferation and differentiation of the responding lymphocytes. Both events can be induced consecutively and *in vitro* by distinct signals¹⁷. The appearance of endogenous viral antigens in dependence of one or both events can thus be studied in detail.

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Are endogenous C-type viruses involved in the immune system?

DNA sequences coding for infectious C-type viruses (oncornaviruses) are present in the genome of normal cells of various species, those best characterised being chicken, mouse, cat and baboon¹. Mammalian endogenous viruses, in general, are restricted for growth in their autologous species but replicate in autologous species and are termed xenotropic². In mice and some other species a further class of endogenous viruses has evolved which is restricted in heterologous cells but replicates in homologous cells, they are termed ecotropic³. Endogenous viral genes are inherited in the germ line and have co-evolved, in general, with non-viral host genes as indicated by evolutionary data⁴. One explanation for the retention of these genes is that they are involved in physiological functions as proposed by Temin in his provirus hypothesis⁵. We have shown previously that B-cell proliferation induced by certain B-cell mitogens is frequently associated with expression of endogenous virus⁶⁻¹⁰. This has been confirmed by other workers who reported a xenotropic host range of the mitogen-induced virus¹¹. In contrast to other virus induction methods, mitogen stimulation closely resembles a physiological process, that of antigen stimulation followed by lymphocyte proliferation. Whereas B cells can be induced by mitogens to release virus, we have evidence that T cells are refractory to induction when using T-cell mitogens as well as 5-bromo-2'-deoxyuridine⁸. These results suggest that the expression of endogenous xenotropic C-type viral genes

may be physiologically required for B cells to participate in the immune response. As a test of this hypothesis we examined the effect of antisera directed against xenotropic endogenous C-type virus on the humoral immune response of mice. We show here that such sera are immunosuppressive.

To obtain an antiviral serum with no activity against non-viral mouse cellular components, we immunised rabbits with mouse xenotropic C-type virus produced in a rabbit cell line (SIRC). This rabbit line had been infected with endogenous C-type virus induced from BALB/c spleen cells by the B-cell mitogen lipopolysaccharide and was used as virus source 8 months after infection. For absorption controls, the same virus was purified from the culture fluid of a similarly infected mink cell line (CCL-64). The sera used in these studies were obtained from two rabbits following multiple intramuscular injections each of approximately 0.2 mg viral protein emulsified in complete Freund's adjuvant. Sera taken from the same animals before immunisation were used as controls. Sera was complement inactivated by heating at 56 °C for 30 min. The immune sera displayed complement-dependent cytotoxicity on virus-infected SIRC cells and showed no activity on uninfected SIRC cells. Pre-immune sera were not cytotoxic.

We examined the effect of antiviral sera on the humoral immune response to sheep red blood cells (SRBC) as assayed in the Mishell-Dutton *in vitro* system¹². In this assay B cells which secrete antibody against SRBC are quantitated by their plaque-forming ability. A typical experiment is presented in Table 1 which shows that anti-serum but not control serum reduces the number of plaque-forming cells by 70%.

To confirm the viral specificity of the immunosuppressive effect of our antisera, absorption experiments were performed with equal quantities of various viruses as well as with SRBC. Table 2 shows that absorption of the anti-serum with xenotropic virus fully removed the immunosuppressive activity. Xenotropic BALB/c virus, grown in mink or rabbit cells, as well as xenotropic AKR virus, were similarly effective in absorption. Some absorption occurred also with ecotropic AKR virus, while tobacco mosaic virus, a completely unrelated virus, showed no absorption. Since rabbit antisera frequently contain activity against Forssman antigen, we tested whether absorption with red cells would remove the immunosuppressive activity. As shown in Table

Table 1 *In vitro* immunosuppressive activity of antiserum against mitogen-induced xenotropic C-type virus

Serum (final dilution)	PFC per 10 ⁷ spleen cells*	% Inhibition
—	3,900	—
Control serum (1:100)	3,900	0
Antiserum		
(1:100)	1,200	70
(1:400)	1,900	51
(1:1,600)	1,700	56
(1:6,400)	3,800	3

The effect of the antiserum against xenotropic C-type virus on the induction of an antibody response to sheep red blood cells was investigated in cultures of BALB/c spleen cells from 8-15-week-old mice by a modified Mishell-Dutton technique¹². 8×10^6 spleen cells were cultured in 35×10 -mm disposable tissue culture Petri dishes (Falcon) containing 1 ml RPMI 1640 medium (Microbiological Associates), supplemented with 8% foetal bovine serum, 1% horse serum and antibiotics. At the onset of the culture period 10 μ l control serum or antiserum was added. The cultures were rocked during the 5-d culture period in an incubator containing 10% CO₂, 83% N₂ and 7% O₂. They were stimulated with 4×10^6 sheep red blood cells. Direct plaque-forming cells (PFC) were assayed by the local haemolysis technique in liquid medium¹³. The cell recovery of the various cultures did not differ significantly.

*Mean values from triplicate cultures.

Table 2 Specificity controls by absorption experiments

Expt no.	Antiserum added	Antiserum absorbed with	PFC per 10 ⁷ spleen cells	% Inhibition
1	—	—	5,200	—
	+	—	800	85
	+	x-BALB/c*	5,200	0
	+	x-AKR†	4,200	19
	+	e-AKR‡	2,200	58
	+	SRBC	1,200	77
2	—	—	1,400	73
	+	—	2,700	—
	+	—	500	82
	+	x-BALB/c*	2,300	15
	+	e-AKR‡	1,300	52
	+	TMV§	500	82
3	—	—	5,800	—
	+	—	600	90
	+	x-BALB/c*	5,600	3
	+	TMV§	1,000	83
	+	SRBC	800	86
	+	Rabbit RBC	600	90

The antiserum was absorbed with either virus or red blood cells. Virus absorption: 400 µg of density-gradient purified virus was pelleted by centrifugation and incubated with 30 µl of sera diluted 1:10 for 60 min in ice. Serum was recovered by centrifugation and 10 µl of the absorbed serum was added to 1 ml cultures. Absorption with red blood cells: 1 part packed cell volume was incubated with 2 parts of sera diluted 1:2 for 30 min on ice. Serum was recovered by centrifugation and subjected to two further absorption procedures. Finally, the serum was clarified by centrifugation in a Sorvall SS 34 rotor at 5,000 r.p.m. for 10 min. Cultures received 10 µl of the absorbed sera or 10 µl of the non-absorbed serum 1:10 diluted. Culture conditions as Table 1.

*Mitogen-induced endogenous xenotropic BALB/c virus, grown in mink CCL-64 cells.

†Mitogen-induced endogenous xenotropic AKR virus, grown in mink CCL-64 cells.

‡Endogenous ecotropic AKR virus grown in mouse NIH-3T3 cells.

§Tobacco mosaic virus.

As *, but virus grown in rabbit SIRC cells.

2, this was not found to be the case. Furthermore, no absorption occurred when the serum was incubated with rabbit SIRC cells (data not shown).

The immunosuppression was also shown in experiments carried out *in vivo*. Four days after immunisation with SRBC those mice which had simultaneously received an intravenous injection of 0.2 ml antiviral serum showed a reduced number of plaque-forming cells, inhibition ranging from 75 to 84% (Table 3). Those mice injected with control serum showed no reduction of plaque-forming cells.

In further experiments the immunosuppressive activity of these sera was also demonstrated with a second antigen, horse red blood cells, which is serologically non-cross-reactive with SRBC. The immunosuppressive activity was found to be due to immunoglobulin since incubating the sera with goat anti-rabbit Ig-serum removed the activity (data not shown).

The possibility that antibody directed against a non-viral cellular antigen is responsible for the observed immunosuppressive effect is highly unlikely for several reasons. As the rabbit antisera did not react with normal rabbit SIRC cells by cytotoxicity or by fluorescence, a contaminating antigen of rabbit origin is unlikely. A mouse cellular antigen is ruled out as the only mouse antigens present in the infected rabbit cell line are viral gene products of the integrated mouse virus (barring transduction of non-viral mouse genes by the virus). Most importantly, virus grown in a third species, mink, also fully removed the activity by absorption.

There seem to be three possible explanations of the mechanism by which our antisera exert this immunosuppressive effect. First, the effect is due to complement-dependent cytotoxicity directed against lymphocytes

Table 3 *In vivo* immunosuppressive activity of antiserum against mitogen-induced xenotropic C-type virus

Expt no.	Serum (dilution) injected	PFC per 10 ⁷ spleen cells*	% Inhibition
1	Control serum	38,400 ± 1,700	—
	Antiserum	9,450 ± 540	75
2	None	28,900 ± 1,400	—
	Antiserum	5,950 ± 470	79
	1:2	4,430 ± 520	84
	1:4	5,480 ± 350	81
	1:8	8,480 ± 480	71
	1:16	11,030 ± 650	61

BALB/c mice aged 8–15 weeks were immunised by one i.p. injection of 5×10^8 SRBC. Antiserum or control serum (0.2 ml per mouse) was injected intravenously immediately after antigen administration. The animals were killed after 4 d and direct plaque forming cells (PFC) were assayed from spleen cells.

*Average of quadruplicate values from spleens of two individual mice. Values are given ± s.e.m.

participating in the anti-SRBC response. This seems unlikely, as the sera were not cytotoxic for spleen cells in a cytotoxic test using rabbit complement. Cytotoxicity on the level of the plaque-forming cells or its precursors also seems unlikely since the immunosuppressive activity was only observed when the antiserum was injected within 2 d after SRBC injection. Later injections during the 4-d immunisation period had no effect. A second possibility is that viral antigen is in close proximity to a membrane structure necessary for the immune response, for example, a receptor. Binding of antibody would then lead to steric hindrance of the receptor function. Finally, viral antigen itself may be required on the membrane. Here it could, for example, play a receptor-like role in cell-cell interaction. Blocking of the antigen therefore would lead to immunosuppression. While this last explanation is consistent with our working hypothesis, it is clear that further studies are necessary if a functional link between endogenous C-type viruses and the immune system is to be established.

These results show that antibody directed against endogenous xenotropic C-type viral antigens represents a new and interesting principle of immunosuppression. Work to identify the target cells of the antisera, the nature of the viral antigen and the mechanism of the immunosuppressive effect is in progress.

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Development of improved cholera vaccine based on subunit toxoid

THE cholera vaccines now available consist of killed vibrios, and give rise to only limited protection of short duration¹. The finding that the excessive diarrhoea in cholera is due to the action on the small intestine epithelium of an exotoxin produced by the vibrios^{2,3} has focused attention on the potential of toxoid for improved immunoprophylaxis. The development of a suitable toxoid has met with great difficulties, however. Formalin toxoid, although reasonably antigenic, proved unsatisfactory because of reversion to toxicity⁴. Glutaraldehyde toxoid was stable but poorly immunogenic which explains its low efficacy in a recent field trial⁵. We describe here the development and properties of a subunit cholera toxoid, the nature of which eliminates the risk of reversion to toxicity. The high protective immunogenicity observed in experimental animals gives promise that addition of this toxoid to the conventional vaccine will result in a significantly improved immunoprophylactic agent against cholera.

Different *Vibrio cholerae* strains, irrespective of their serotype, produce an immunologically identical exo-enterotoxin⁶. The toxin contains two types of non-covalently linked subunits, one heavy (H or A; molecular weight (MW) about 28,000) and five or six light ones (L or B, MW about

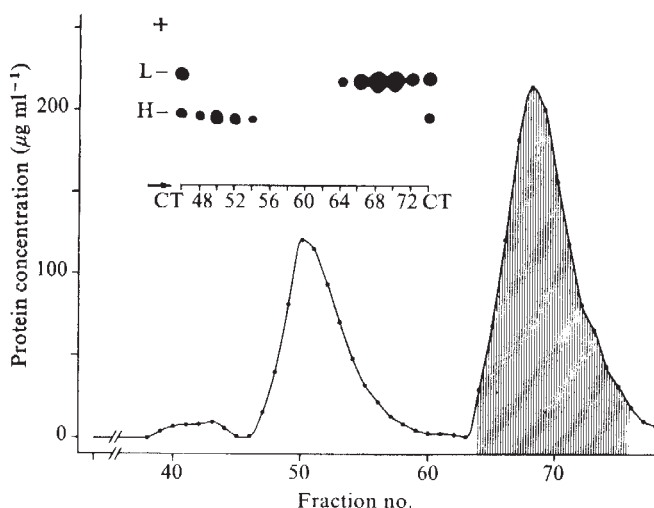


Fig. 1 Preparation of L-subunit toxoid from purified cholera toxin. The toxin (about 10 mg in 1–2 ml) was diluted with 20% concentrated formic acid to give a 5% final concentration of the acid. After incubation for 1 h, the sample was filtered (at room temperature) through a 2.5 × 87.5-cm Sephadex G-100 column equilibrated as well as eluted (25 ml h⁻¹) with 5% formic acid. Consecutive 4-ml fractions were collected and their light absorbance at 280 nm determined. The diagram shows the protein elution profile from a typical experiment, in which 8.5 mg toxin was processed. Two distinct peaks emerge, the first representing isolated subunit H and the second, bigger one subunit L. The amounts of applied toxin and eluted subunits were estimated from the absorbance values using the $A_{280}^{1\%}$ coefficients¹ 11.4 for the toxin, 15.1 for subunit H and 9.56 for subunit L fractions. The fractions hatched in the diagram were pooled, dialysed against PBS (0.05 M phosphate, 0.14 M sodium chloride, pH 7.2) at 4°C for 48 h, and then used as L-subunit toxoid. The inserted photograph illustrates the results obtained on testing samples of individual gel filtration fractions by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). The samples were lyophilised, redissolved in 2% SDS-8M urea solution, incubated at 53°C for 20 min and then processed by SDS-PAGE⁷. Every second fraction from number 48 to 72 was tested. In addition, cholera toxin (CT) was included in two positions as a reference for the migration of subunits H and L. The arrow indicates start line and + the anode direction.

Table 1 Protective immunity and serum antibody titres in rabbits after immunisation with L-subunit and glutaraldehyde toxoids

Dose of toxoid (μg)	No. of animals	Protection factor Live vibrios	Crude toxin	Serum antitoxin titre
L-subunit toxoid				
30	7	18	5.0	400,000
15	4	10	2.5	130,000
10	8	3.2	1.8	100,000
7.5	5	1.3	1.4	25,000
Glutaraldehyde toxoid				
30	4	5.0	1.6	20,000
15	4	0.7	1.1	10,000

New Zealand rabbits weighing 1.2–1.5 kg at the onset of immunisation were used. The L-subunit toxoid was prepared as described in Fig. 1. The glutaraldehyde toxoid²¹ (Wyeth; lot 20101) was provided by Dr C Miller of NIH. The antigens were injected s.c. without adjuvant in two 0.5 ml portions above the right and left posterior legs twice with a 2-week interval. A similar number of control rabbits as in the immunised group were injected with phosphate-buffered saline (PBS). Five days after the booster injection the immunised and control animals were tested for susceptibility to experimental cholera. In each animal 21 about 5-cm long segments of the small intestine were ligated. Into each loop was injected graded doses (in 1-ml volumes) of live cholera vibrios (strain 569B) or crude cholera toxin (*V. cholerae* 569B culture filtrate, NIH lot 4493G). Five doses of bacteria (10^5 – 10^9) and crude toxin (0.1–10 mg) were tested in duplicate at randomly chosen positions. The remaining loop was injected with 1 ml PBS and served as a negative control. Fluid accumulation in the intestinal segments was measured after 15–18 h as ml fluid per cm intestine and ED₅₀ (dose of bacteria or toxin producing half-maximal intestinal filling) determined for each group of animals²⁰. The protective effect of immunisation—the protection factor—was estimated as the ratio between the group ED₅₀ values for immunised and concurrently tested control animals. Serum antitoxin titres were determined by the enzyme-linked immunosorbent assay (ELISA)²² on serum samples, pooled in each group, collected on the day of the intestinal challenge.

11,000 each)^{7–12}. The L subunits are responsible for the binding of toxin to the cell membrane but not for the subsequent activation of adenylate cyclase. Conversely, the H subunit is essential for toxicity but, because of its inability to bind to cells, it is inactive in the absence of L subunits^{13,14}. The two types of cholera toxin subunits are immunologically unrelated¹³. The subunits also differ markedly in immunogenicity so that immunisation with intact toxin gives rise to antisera which always react strongly with the L subunit but only irregularly and at low titre with subunit H. Furthermore, isolated anti-H subunit antibodies have very low cholera toxin-neutralising capacity in contrast to antibodies reacting with subunit L¹⁵. Immunological protection by means of cholera antitoxin thus probably results from prevention of toxin binding to intestinal cell receptors rather than from interference with the 'active site' on the effector subunit. We have therefore directed our efforts to the preparation of pure L subunits and reaggregation of these subunits. Theoretically this should yield an almost ideal toxoid for immunisation, since the elimination of subunit H excludes the risk of reversion to toxicity without causing any significant loss of protective capacity.

The method for preparation of L-subunit toxoid was based on the fact that the two types of subunit in cholera toxin can be isolated by gel filtration of toxin in acidic buffer⁷. Highly purified toxin, prepared essentially according to Finkelstein *et al.*¹⁶, was treated with formic acid and the dissociated subunits separated on Sephadex G-100 (Fig. 1). This resulted in an almost quantitative isolation of the toxin L subunits in pure form. In five different experiments the yield of L was 90–93% and contamination with subunit H could be excluded at a level of 0.2% (w/w) using sensitive immunological methods. As determined with the rabbit skin¹⁷ and mouse thymocyte cyclic AMP accumula-

Table 2 Increased duration of protection by immunisation with toxoid or toxoid-enriched cholera vaccine

	Protection factor				Serum antibody titres			
	Day 5		Day 21		Day 5		Day 21	
	Live vibrios	Crude toxin	Live vibrios	Crude toxin	LPS	Toxin	LPS	Toxin
Cholera vaccine (4×10^9 killed vibrios)	10	1.1	2.0	1.0	120,000	< 500	300,000	< 500
L-subunit toxoid (10 μ g)	3.2	1.8	13	3.0	< 500	100,000	< 500	200,000
Cholera vaccine + L-subunit toxoid	80	2.1	90	3.0	100,000	400,000	500,000	600,000

Groups of 8–12 rabbits were immunised according to the schedule described in Table 1. Twelve concurrently PBS-injected rabbits served as controls. Half of the animals in each group were challenged with live vibrios or crude toxin in alternating loops 5 d and the others 21 d after the booster immunisation. The protective effect of immunisation (the protection factor) and the serum antibody titres against *V. cholerae* lipopolysaccharide (LPS) and enterotoxin were determined as in Table 1.

tion assays¹⁸, all batches of L-subunit toxoid had less than 0.1% of the toxicity of intact toxin.

Radial immunodiffusion tests (Mancini) suggested that the L subunits in the toxoid, with retention of their full reactivity with antibody, were aggregated to a complex with a diffusion rate similar to that of native toxin. This aggregation was demonstrated directly by thin-layer gel filtration in Sephadex G-200 superfine¹⁹. The L-subunit toxoid migrated as a single component with a rate faster than that of subunit H and there was no detectable toxoid protein filtering at the rate which would be expected for the individual L subunits.

The toxoid was usually kept frozen in aliquots at -30°C , and storage for more than 18 months did not result in detectable changes according to the immunological, physicochemical and biological criteria mentioned above. In the presence of 0.01% merthiolate the toxoid has been stable at 4°C – 8°C for more than 6 months.

The immunogenicity of the L-subunit toxoid was studied in rabbits and compared with that of the recently field tested glutaraldehyde toxoid. Two subcutaneous (s.c.) immunisations with 10 μ g of L-subunit toxoid in each injection induced significant protection (Table 1). The effect in-

creased with increasing amounts, so that 30 μ g gave an approximately 20-fold increased resistance to live vibrio challenge and 5-fold protection against challenge with toxin. The glutaraldehyde toxoid was much less effective and also gave rise to lower serum antibody titres than the L-subunit toxoid (Table 1). Adsorption to aluminium phosphate did not significantly enhance the protective immunogenicity of the L-subunit toxoid as tested with 10 μ g of toxoid on 0.034 mmol of carrier. No side-effects of immunisation were observed in any instance.

V. cholerae enterotoxin and cell wall lipopolysaccharide (LPS) has been shown to induce a synergistic protective immunity against experimental cholera infection²⁰. In agreement with this, combinations of L-subunit toxoid and conventional cholera vaccine or purified LPS were found to induce a level of protection against intestinal challenge with live vibrios which equalled or exceeded the product of the immunity attained with the individual antigens (Fig. 2). Challenge in alternate loops with crude toxin (not shown) revealed that the somatic antigens neither gave rise to any antitoxic immunity nor enhanced the efficacy of the toxoid component in protection against toxin. Furthermore, the serum antibody titres induced by the antigens in the mixture

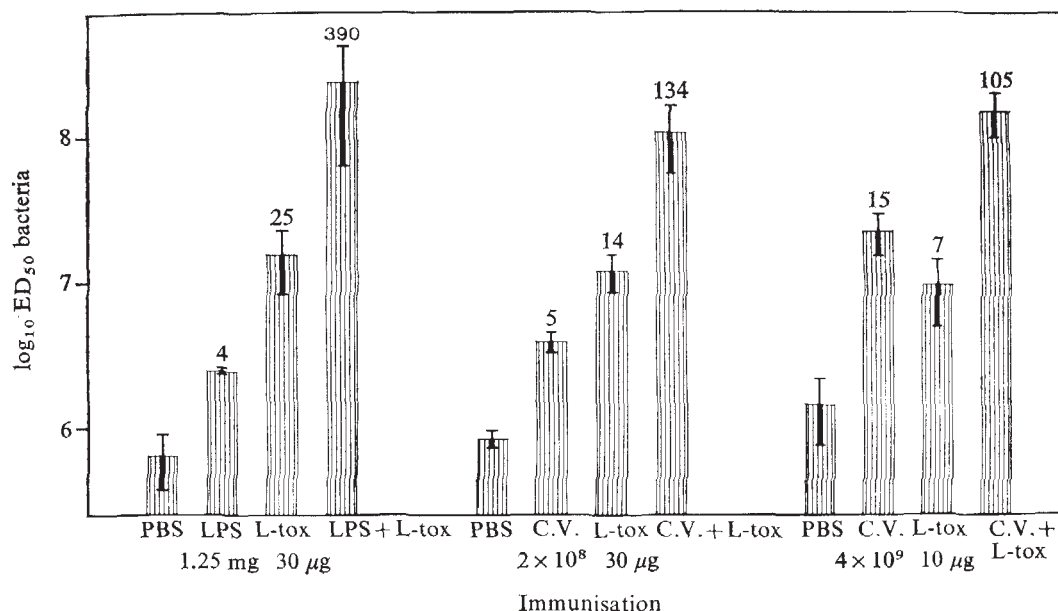


Fig. 2 Synergistic protective effect of immunisation with combinations of *V. cholerae* somatic antigen and L-subunit toxoid. Three experiments are presented, each comprising four groups of four to eight rabbits. One group received PBS injections only, and the others were immunised according to the schedule described in Table 1 with, respectively, somatic antigen (killed-*Vibrio cholerae* vaccine (C.V.)) in two doses, or purified *V. cholerae* lipopolysaccharide (LPS), L-subunit toxoid (L-tox.), or the somatic antigen and toxoid in mixture. Five days after immunisation intestinal challenge with graded doses of live vibrios was performed as described in Table 1 and ED_{50} was determined for each animal. The means $\pm$ s.e.m. of the individual ED_{50} values of bacteria are shown for each group of rabbits. The figures on top of the bars are the ratios between the mean ED_{50} for immunised animals and the mean ED_{50} for the PBS-injected control rabbits, that is, the fold-increase protection achieved by immunisation. The cholera vaccine (National Bacteriological Laboratory, Stockholm) contained equal numbers of phenol-killed Inaba and Ogawa vibrios. The LPS was purified from *V. cholerae* 569B by means of hot phenol-water extraction and ultracentrifugation²³.

did not differ significantly from those produced by the respective antigens alone. These data indicate that the synergistic protective effect obtained by immunisation with toxoid and somatic antigen in combination is not due to enhancement of the antitoxic and antibacterial immune responses in themselves. Instead, it probably results from the interference of the immunity with two separate events of pathogenic importance, that is, the mucosal adhesion of cholera vibrios and binding of enterotoxin to specific membrane receptors¹⁵.

As in humans, the conventional vaccine gave rise only to short-term protective immunity in rabbits. The immunity against challenge with live vibrios decreased markedly between day 5 and 21 after the booster injection, in spite of a slight increase in the serum anti-LPS antibody titre (Table 2). In contrast, a combination of L-subunit toxoid and vaccine gave rise to immunity which rather increased during this period. This was probably due to a sufficient rise in the antitoxic immunity induced by the toxoid component to compensate for the rapid decrease in antibacterial immunity, as immunity induced by L-subunit toxoid alone increased substantially from day 5 to 21 (Table 2).

In conclusion, a subunit cholera toxoid having no risk of reverting to toxicity has been developed. Vaccination with a mixture of this toxoid and killed vibrios should result in more effective, longer lasting immunity against cholera than that given by immunophylactic agents used previously. Studies in progress suggest that inexpensive large-scale production may be possible by preparing the subunit toxoid from crude toxin preparations.

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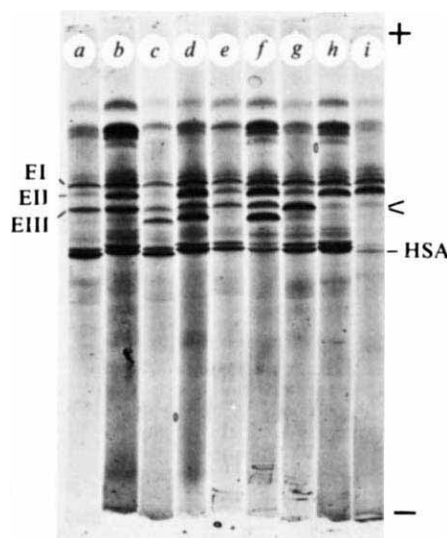
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Polymorphism of apolipoprotein E and occurrence of dysbetalipoproteinaemia in man

PRIMARY dysbetalipoproteinaemia (broad- β disease, hyperlipoproteinaemia type III) is a familial disorder of plasma lipoprotein metabolism associated with xanthomatosis and early onset of severe atherosclerotic vascular disease^{1–4}. A defect in the catabolism of triglyceride-rich lipoproteins probably underlies the accumulation of atypical cholesterol-rich lipoproteins (very low density β lipoproteins, ' β -VLDL') in the S_f 12–400 density fraction^{5–7}. The disorder is assumed to be rare⁸ and we have provided evidence for an autosomal recessive mode of inheritance^{9,10}. Patients show a variant of one major protein component (Apo E) of VLDL^{11,12}. Apo E is a glycoprotein of molecular weight $\sim 39,000$ that splits into three main bands designated E-I ($pI \sim 5.3$), E-II ($pI \sim 5.4$) and E-III ($pI \sim 5.55$) in isoelectric focusing¹³. The Apo E variant is characterised by a deficiency of Apo E-III in triglyceride-rich lipoproteins. We report here that Apo E shows a genetic polymorphism determined by two alleles *Apo Eⁿ* and *Apo E^d*. About 1% of the German population is homozygous for the allele *Apo E^d* and exhibits Apo E-III deficiency. All individuals of this genotype have a primary dysbetalipoproteinaemia but not necessarily hyperlipidaemia. This is therefore the most frequent monogenic dyslipoproteinaemia known in man.

A simple method for the detection of Apo E patterns without ultracentrifugation that allows the selective recognition of patients with dysbetalipoproteinaemia in a hyperlipidaemic group (refs 9, 11 and unpublished work of G.U., M.H., G. Mühlfellner, N. Pruin and K. H. Vogelberg) has been applied to evaluate Apo E patterns in 490 blood donors from Marburg/Lahn. VLDL was precipitated from 1 ml of serum by addition of 50 μ l of a 5% aqueous heparin solution and 50 μ l 2 M MgCl₂. Crude VLDL was isolated by low-speed centrifugation, resuspended in 0.5 ml 0.02 M Tris-HCl, 0.1 M NaCl, 0.017 M sodium citrate pH 7.7 and reprecipitated by addition of 25 μ l 2 M MgCl₂. VLDL was extracted with chloroform-methanol, 2 : 1 (v/v). Apo VLDL was solubilised

Fig. 1 Analytical isoelectric focusing (pH 3.5–10 in polyacrylamide gels containing 6 M urea) of nine individual apo-VLDL preparations demonstrating the different Apo E phenotypes Apo E-N (a, c, g), Apo E-ND (b, d, e, f) and Apo E-D (h, i). The additional cathodic band ($pI \sim 5.75$) in gels c, d, f represents a further independent genetic marker (Apo E-IV(+)-variant) that had a frequency of 26.9% in the population sample studied and is transmitted as an autosomal dominant trait¹⁷ (see also Fig. 3).



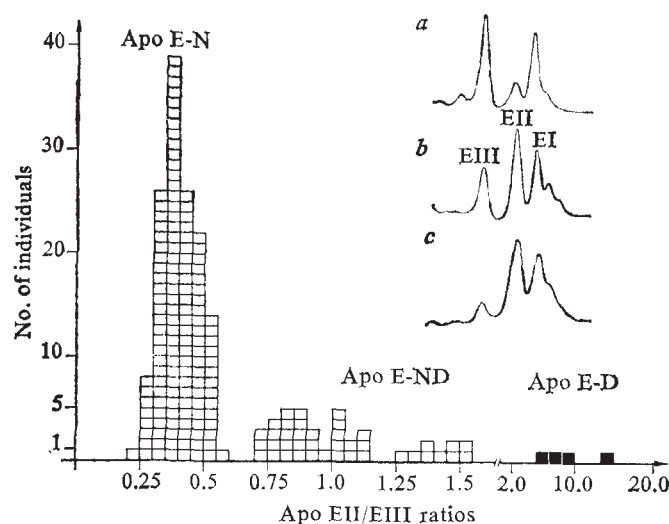


Fig. 2 Distribution of Apo E patterns according to Apo E-II/E-III ratios (densitometric areas) in individual apo VLDL samples ($n=181$) that have been evaluated by scanning densitometry. Each square represents one individual. Probands with features of dysbetalipoproteinaemia are marked in black. Insert: representative densitometric scans of stained focusing gels demonstrating the three common Apo E phenotypes Apo E-N (a), Apo E-ND (b) and Apo E-D (c). Only the Apo E region is shown. The pI values of the individual bands determined by flat bed isoelectric focusing in granulated gels were ~ 5.3 (E-I), ~ 5.5 (E-II) and ~ 5.55 (E-III). (U. Beisiegel and G.U., unpublished) and differed slightly from those determined in the analytical polyacrylamide gels¹³.

in 200 μ l 0.02 M Tris-HCl, 8 M urea, pH 8.2 and subjected to isoelectric focusing in a pH gradient from 3.5–10 in polyacrylamide gels containing 6 M urea. Protein bands were stained with coomassie brilliant blue and evaluated by scanning densitometry.

Three phenotypes, Apo E-N, Apo E-ND and Apo E-D (E-III deficiency pattern) were differentiated on the basis of the Apo E-II/E-III ratios (Figs 1 and 2). The ratio was 0.40 ± 0.07 for phenotype Apo E-N, 1.02 ± 0.25 for phenotype Apo E-ND and 9.07 ± 4.48 for phenotype Apo E-D. No overlap was observed between the distinct groups in this study.

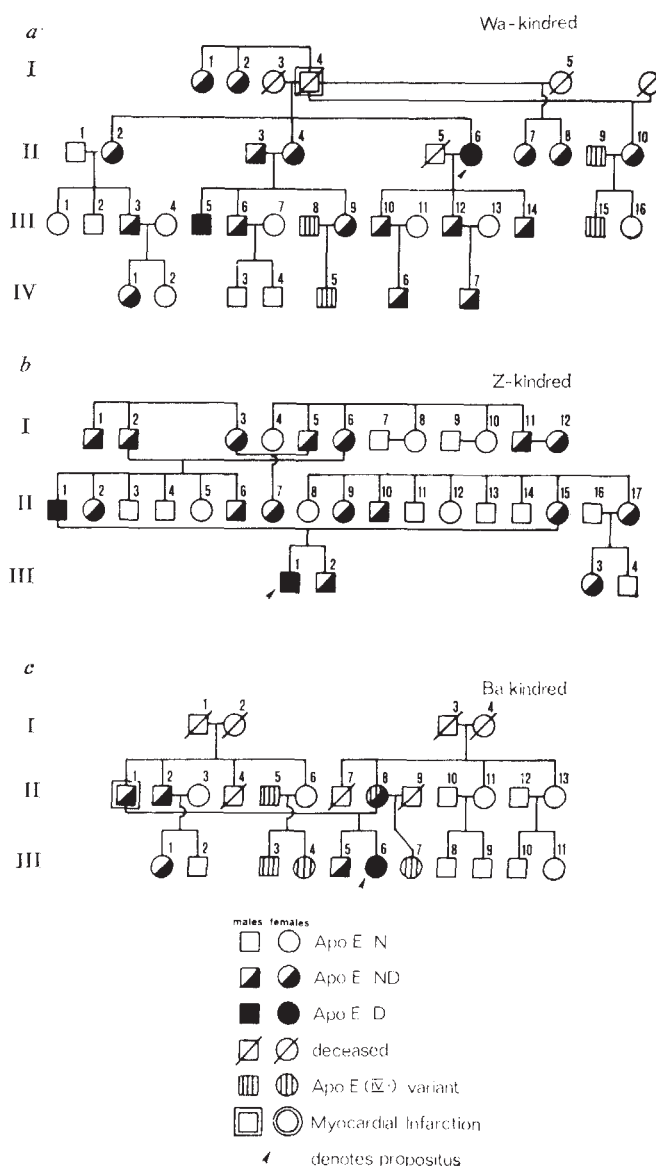
In control experiments VLDL was isolated by conventional preparative ultracentrifugation from individuals of phenotypes Apo E-D ($n=5$) and Apo E-ND ($n=51$) and from a control group of the same size. All individuals were in a fasting state. In all cases the previous diagnosis was confirmed. The demonstration of Apo E phenotypes is therefore independent of the method used for isolation of VLDL. The frequencies of the three common Apo E phenotypes were 83.3% for Apo E-N, 15.7% for Apo E-ND and 1.0% for Apo E-D.

Apo E patterns were determined in three kindreds of probands of phenotype Apo E-D (Fig. 3). The segregation of Apo E phenotypes among children from different parental matings is consistent with a simple Mendelian mode of transmission where two alleles determine the three phenotypes Apo E-N, Apo E-ND and Apo E-D. Considered together, the population and family data show that, in man, genetic polymorphism of apolipoprotein E is governed by two common autosomal alleles *Apo Eⁿ* (frequency 0.9112) and *Apo E^d* (frequency 0.0888). The observed frequencies of the three phenotypes are in good agreement with those expected according to Hardy-Weinberg's law. No significant differences between females ($n=144$; *Apo Eⁿ*=0.9201) and males ($n=346$; *Apo Eⁿ*=0.9075) were observed.

The frequency of the allele *Apo E^d*, however, was unexpectedly high. We therefore investigated whether the

homozygous probands of phenotype Apo E-D detected had dysbetalipoproteinaemia. Analysis of the composition of the main lipoprotein fractions of all five probands in comparison with a control group of 94 subjects of phenotypes Apo E-N ($n=43$) and Apo E-ND ($n=51$) showed that they in fact represented a biochemically distinct group with abnormalities in their lipoprotein spectrum characteristic of dysbetalipoproteinaemia. These were (1) an abnormal quantitative distribution of lipoproteins (demonstrated by density gradient centrifugation) with reduced levels of LDL and elevation of VLDL and also intermediary density lipoprotein levels (Fig. 4); (2) a markedly reduced or absent LDL band in polyacrylamide gel electrophoresis (Fig. 5); (3) presence of ' β -VLDL' in the $d < 1.006$ g ml⁻¹ fraction in the ultracentrifuge (Fig. 5) and (4), an increase in the relative and absolute concentrations of cholesterol in VLDL (Table 1). Thus all probands had dysbetalipoproteinaemia according to the 'floating- β test' and also according to at least one of the lipid-chemical definitions recently proposed for the disorder^{14,16}. However, none had hypercholesterolaemia and only three had mild hypertriglyceridaemia: total serum cholesterol levels

Fig. 3 Pedigrees of three kindreds demonstrating the segregation of Apo E phenotypes. Propositi were a A.W. (Wa-kindred); b, B.Z. (Z-kindred) and c, M.B. (Ba-kindred) (see Table 1).



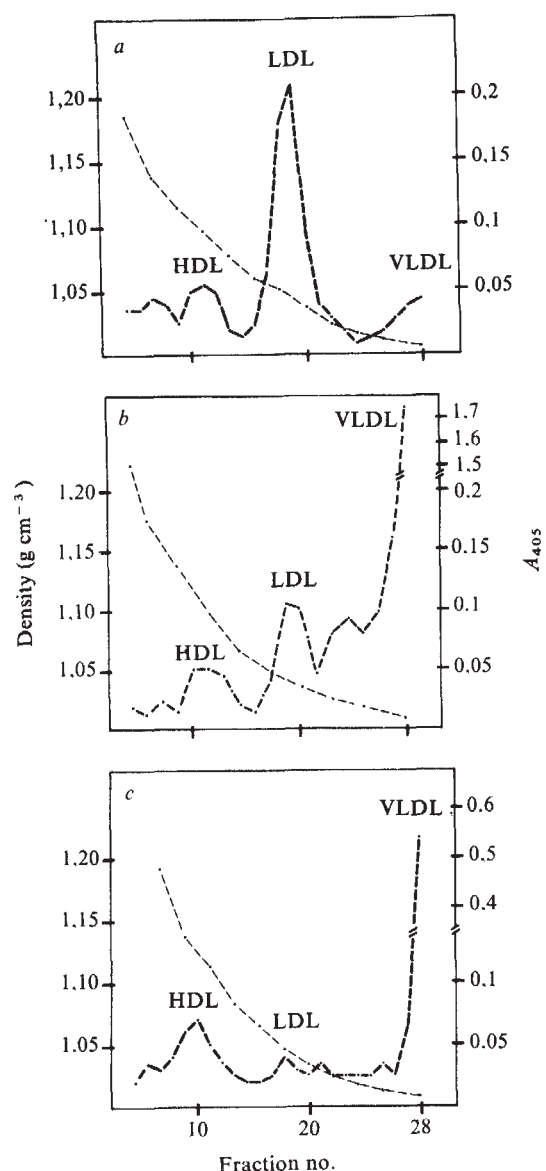


Fig. 4 Profiles of lipoprotein cholesterol after density gradient centrifugation of 4 ml of serum from a normolipidaemic control subject (a), a hyperlipidaemic proband of phenotype Apo E-D (b), and a normocholesterolaemic proband of phenotype Apo E-D (c). Centrifugation was performed in a SW-41 rotor at 40,000 r.p.m. for 24 h at 4 °C. The gradient was from 1.006 g ml⁻¹ to 1.21 g ml⁻¹ KBr and prepared according to Redgrave *et al.*¹⁸. Fractions were collected from below and cholesterol determined with the enzymatic method of Roeschlau¹⁹. — — —, Absorbance at 405 nm of 3,5-diacetyl-1, 4-dihydrolutidine the endproduct in the enzymatic test for cholesterol. — . — . — ., Density.

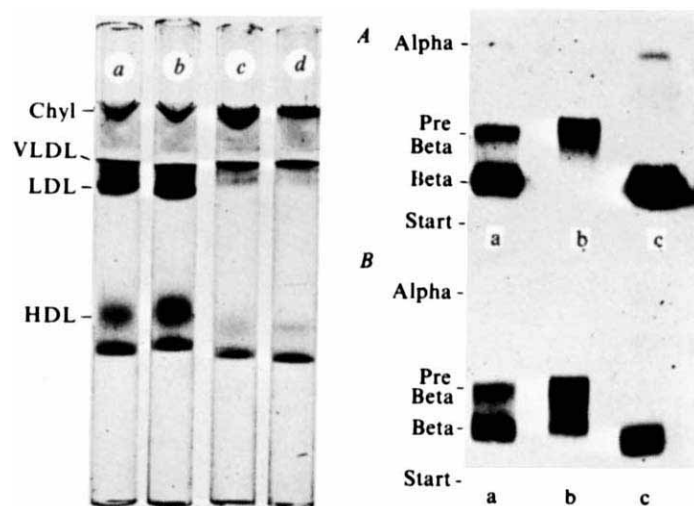


Fig. 5 Left: Discontinuous electrophoresis in polyacrylamide gels of 3.75% monomer concentration of sera prestained with sudan black B²⁰ from two controls of phenotype Apo E-N (a, b) and two probands of phenotype Apo E-D (c, d). The poor staining of the HDL-band in gels c and d indicated a decrease in the concentration also of this component but was not seen in all individuals of phenotype Apo E-D. Chyl, Chylomicrons. Right: Agarose gel electrophoresis of sera (a), VLDL (b) and fraction d > 1.006 g ml⁻¹ (c) from a control subject (A) and a proband of phenotype Apo E-D (B). Staining sudan black B.

were abnormally low in the three probands under age 30 yr. From a study of subjects with primary hypocholesterolaemia where about 10% were of phenotype Apo E-D (compared with 1% in the general population observed in this study) we have additional evidence that young individuals of phenotype Apo E-D represent a novel genetic form of hypocholesterolaemia with hypobetalipoproteinaemia (G.U., H. Kaffarnik, and N. Pruin, in preparation). According to the data presented here the gene frequency for dysbetalipoproteinaemia (including hypo-, normo- and hypercholesterolaemic subjects) is 0.089 in the German population sample studied. This high frequency explains the occurrence of vertical transmission of dysbetalipoproteinaemia^{2,4,8} as pseudo-dominance (see Z-kindred in Fig. 2 and refs 9, 10).

In contrast to the high frequency of dysbetalipoproteinaemia in the population, individuals of phenotype Apo E-D that have gross hyperlipidaemia and xanthomatosis seem to be rare. Studies in progress in our laboratory indicate that additional mutant genes for other forms of familial dyslipoproteinaemia (familial combined hyperlipidaemia, familial hypertriglyceridaemia, familial hypercholesterolaemia) are operating in those patients that have

Table 1 Clinical data on the five probands of phenotype Apo E-D

Subject	Age/sex	Serum		VLDL*		VLDL _{Ch/TG}	Ch _{VLDL} /TG _{serum}	β-VLDL
		Cholesterol (mg per 100 ml)	Triglycerides (mg per 100 ml)	Cholesterol (mg per 100 ml)	Triglycerides (mg per 100 ml)			
B.Z.	24/M	89	77	23	61	0.38	0.3	+
B.Th.	26/M	128	157	64	83	0.77	0.41	+
M.B.	28/F	113	78	42	45	0.94	0.54	+
G.W.	60/M	196	141	41	66	0.61	0.27	+
A.W.	65/F	177	175	42	74	0.53	0.24	+
Mean ± s.d.		141 ± 45	128 ± 47	42 ± 15	66 ± 14	0.65 ± 0.22	0.35 ± 0.12	
Controls ± s.d.		191 ± 31	102 ± 34	17 ± 6	59 ± 39	0.32 ± 0.15	0.17 ± 0.07	

A group of six age- and sex-matched blood donors of phenotype Apo E-N served as controls.

*VLDL was isolated from 200–250 ml of serum to get reliable data and lipid values are given as mean of direct and indirect determinations.

developed severe clinical manifestations. One of the most intriguing questions is whether probands of phenotype Apo E-D that have dys- but not hyperlipoproteinaemia are also at risk to develop premature atherosclerosis.

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Human serum lipoproteins activate adipocyte plasma membrane adenylate cyclase

BROWN and Goldstein¹ and Stein *et al.*², have shown evidence for the role of a specific cell surface receptor in the binding and catabolism of serum low-density lipoproteins (LDL) by cultured human fibroblasts. This receptor does not seem to be unique to fibroblasts however, since it has been shown that circulating human lymphocytes³, human fat cells⁴ and cultured smooth muscle cells⁵ may also bind LDL; also, binding sites for LDL and very low density lipoproteins (VLDL) seem to be present in crude membrane preparations from various organs and tissues of the pig⁶. These findings suggest that lipoprotein receptors are ubiquitous in mammalian cells and raise the possibility that occupancy of such surface binding sites might modify some aspect of membrane function. Evidence for an alteration of membrane function by lipoproteins was recently reported by Shore and Shore⁷, who observed that human serum VLDL and LDL were capable of specifically activating the human erythrocyte membrane Mg^{2+} -ATPase.

The plasma membrane of the adipocyte is intimately involved in lipid metabolism, particularly as a result of its role in the hormone-sensitive adenylate cyclase–triglyceride lipase system⁸. Since the properties and enzyme activities of this membrane may be modified by the components of its external environment, it seemed possible that circulating lipoproteins might affect its function. We describe here the activatory effect of human serum lipoproteins on the adenylate cyclase system of plasma membranes from human and rat adipocytes.

Adipocytes were isolated by collagenase digestion⁹ from the epididymal fat pads of male Wistar rats (150–180 g),

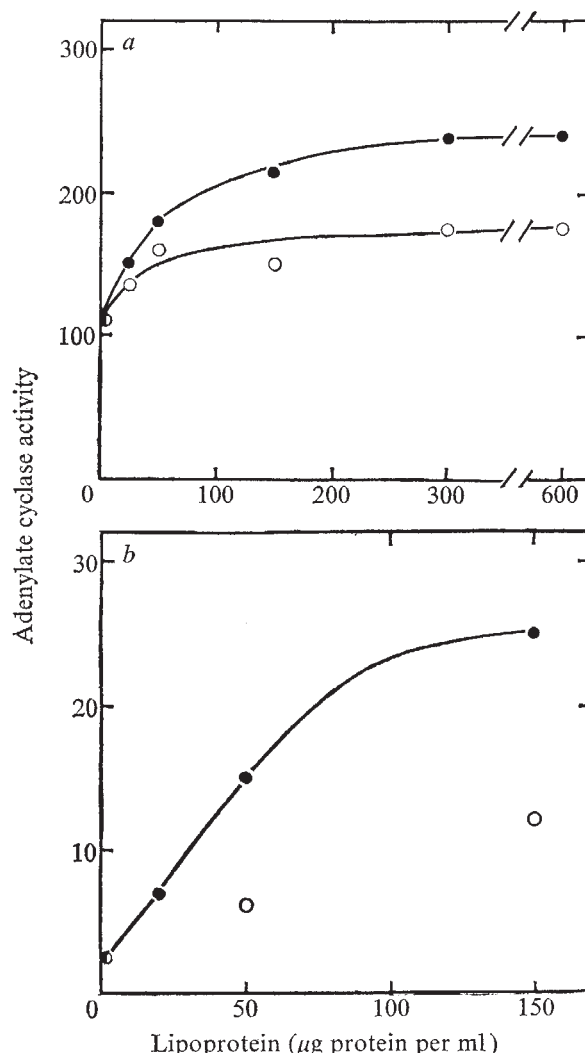


Fig. 1 Adenylate cyclase activity of plasma membranes from rat and human adipocytes as a function of serum lipoprotein concentration. The final incubation medium (50 µl) for the adenylate cyclase assay contained 0.2 mM [α - 32 P]-ATP (0.5 µCi) (Radiochemical Centre, Amersham), 5 mM $MgSO_4$, 1 mM cyclic AMP, 1 mM EDTA, 1 mM dithiothreitol, 50 mM Tris-HCl (pH 7.8), an ATP-regenerating system consisting of 10 mM phosphocreatine (di-Tris salt, Sigma) and 0.5 IU creatine phosphokinase (Calbiochem). Plasma membranes from rat adipocytes (2 µg protein (a)) or from human adipocytes (1.5 µg protein (b)) were added to the reaction mixture (containing 0.2 mM ATP). Preincubation was carried out for 15 min at 30 °C in the presence of LDL (○) or VLDL (●); lipoprotein samples were added in a volume of 10 µl. At the end of the preincubation period, labelled ATP was added and the reaction allowed to proceed for 10 min at 30 °C. The accumulation of labelled cyclic AMP was linear as a function of time. The reaction was terminated according to White¹³ and the 32 P labelled—cyclic AMP was isolated by column chromatography on neutral alumina¹³. Each determination was performed in duplicate. Results are expressed as pmol cyclic AMP formed per min per mg protein. The amount of ATP present at the end of the incubation was determined by thin-layer chromatography on polyethyleneimine-cellulose developed in 1 M LiCl. In these conditions, residual ATP amounted respectively to 92% and 96% of its original concentration, in the absence and in the presence of 260 µg VLDL protein per ml. The product formed in the reaction mixture was identified as authentic 32 P-cyclic AMP by three procedures: (1) it migrated together with authentic cold cyclic AMP on cellulose plates developed in 1 M ammonium acetate and 95% ethanol (1/2.5, v/v); (2) the product of the reaction had the same specific activity when isolated either by the routine method described above or after two chromatographic steps through Dowex AG 50 W-X8 (H^+ form) and neutral alumina¹⁴; and (3) 90% of the 32 P labelled product isolated in the presence or the absence of lipoproteins in the assay medium of adenylate cyclase was hydrolysed by 3', 5'-cyclic AMP phosphodiesterase from beef heart (Sigma, 20 µg; 20 min incubation at 30 °C).

Table 1 Activation by human serum VLDL of adenylate cyclase from rat adipocyte plasma membranes at several ATP and Mg^{2+} concentrations

ATP (μM)	Adenylate cyclase activity (pmol cyclic AMP per mg protein per min $\pm$ s.e.)*					
	1		5		50	
	0	VLDL (200 μg ml $^{-1}$)	0	VLDL (200 μg ml $^{-1}$)	0	VLDL (200 μg ml $^{-1}$)
10	2.8 $\pm$ 0.6	6.6 $\pm$ 0.4 (137)	16.6 $\pm$ 1.2	27.5 $\pm$ 0.9 (66)	13.1 $\pm$ 0.2	20.1 $\pm$ 0.6 (53)
50	6.8 $\pm$ 0.7	13.6 $\pm$ 1.5 (99)	40.2 $\pm$ 1.6	68.5 $\pm$ 1.5 (70)	54.5 $\pm$ 0.1	79.5 $\pm$ 0.1 (46)
200	6.6 $\pm$ 2.2	7.6 $\pm$ 0.6 (15)	61.3 $\pm$ 8.8	96.7 $\pm$ 6.2 (58)	127.8 $\pm$ 2.1	181.5 $\pm$ 0.2 (42)

Rat fat cell membranes (1.5 μg of protein) were incubated in the experimental conditions described in the legend to Fig. 1. The numbers in parentheses represent percentage cyclase activation relative to the basal level.

* Standard error of triplicate determinations.

while human adipocytes were prepared from subcutaneous fat obtained at surgery. Plasma membranes were separated as previously described¹⁰. Lipoproteins were isolated at 5° C from the sera of normolipidaemic adult donors by sequential preparative ultracentrifugation (see Havel *et al.*¹¹); the detailed conditions have been previously outlined¹². The density intervals used for the various fractions were: VLDL, $d < 1.006$ g ml $^{-1}$; LDL, d 1.024–1.045 g ml $^{-1}$ and high-density lipoprotein (HDL), d 1.080–1.210 g ml $^{-1}$. All fractions were extensively dialysed at 5° C against a solution containing 150 mM NaCl, 0.5 mM Na₂EDTA and 5 mM Tris-HCl at pH 7.5.

Human serum VLDL and LDL stimulated the basal activity of adenylate cyclase from human and rat adipocyte plasma membranes (homologous and heterologous systems respectively) in a dose-dependent manner (Fig. 1). Such activation was a saturating phenomenon; half-maximal activation occurred in the concentration range of 50–100 μg VLDL or LDL protein ml $^{-1}$ (Fig. 1). The stimulation of basal adenylate cyclase activity by VLDL was more potent than that elicited by LDL. The effect of HDL on enzyme activity was always inferior to or equal to that of LDL (data not shown).

The degree of activation of the adenylate cyclase system in a given membrane preparation varied with VLDL isolated from different sera. For example, the same human membrane preparation studied with three separate VLDL samples (protein concentration 150 μg ml $^{-1}$), elicited activations amounting to 250%, 1,400% and 1,970% respectively of that of the basal adenylate cyclase activity determined in standard conditions (for details see Fig. 1 legend). Such observations may probably be attributed to variations in the relative proportions of the different particle species present in each VLDL fraction. A more constant but lower degree of stimulation by human VLDL was found in experiments with plasma membranes from rat

adipocytes (78 $\pm$ 13%, mean value $\pm$ s.e.m., n = 6 for 4 membrane preparations), that is, in the heterologous system.

The effect of substrate concentration on cyclase stimulation by VLDL was examined by variation of the ATP concentration in the range 10–200 μM at several Mg^{2+} concentrations in the assay medium. The results indicated a strong substrate (Mg ATP) dependency of the cyclase activation by VLDL which occurred at an Mg^{2+} concentration of 1 mM: at higher Mg^{2+} concentrations, the extent of enzyme activation by VLDL was essentially unchanged in the range 10–200 μM ATP (Table 1).

The possibility that lipoproteins could also modify the cyclic AMP-phosphodiesterase activity in the membrane preparations was investigated. The adenylate cyclase activity measured in the presence of 1 mM cyclic AMP was only 10–15% above that observed in its absence and the extent of cyclase activation by lipoproteins was unaffected by the presence of cyclic AMP. Also, additional experiments indicated that VLDL were without effect on the membrane phosphodiesterases of low and high K_m , whose activities were measured by the method of Thompson and Appleman¹⁵ (data not shown). We conclude therefore that the increased synthesis of ³²P-labelled cyclic AMP which we have observed results specifically from an effect of serum lipoproteins on the adenylate cyclase system.

Activation by serum lipoproteins of adenylate cyclase in both homologous and heterologous experimental systems also occurred when the enzyme was maximally stimulated by 0.1 mM adrenaline (bitartrate), by 10 mM fluoride ion or by 0.1 mM guanylyl (β , γ -methylene)-diphosphonate (Gpp(CH₂)p) (Table 2). These findings show that serum lipoproteins do not seem to modify the responsiveness of the cyclase system to hormones or other effectors; similar effects have been reported in other systems following the addition of polycationic peptides¹⁶ or after limited proteolysis¹⁷. Our present observations are, however, consistent with the sug-

Table 2 Effect of human serum VLDL and LDL on adenylate cyclase activity in human and rat fat cell membranes stimulated by adrenaline, fluoride ion and Gpp(CH₂)p

Plasma membranes	Addition	Adenylate cyclase activity (pmol cyclic AMP per mg protein per min $\pm$ s.e.)*			
		None (basal)	Adrenalin (0.1 mM)	NaF (10 mM)	Gpp(CH ₂)p (0.1 mM)
	0	2.5 $\pm$ 0.5	3.5 $\pm$ 0.6	30.3 $\pm$ 7.2	10.1 $\pm$ 1.8
Human	VLDL	25.8 $\pm$ 1.5	28.6 $\pm$ 3.2	220.0 $\pm$ 22.6	81.0 $\pm$ 0.9
	LDL	11.8 $\pm$ 1.5	16.0 $\pm$ 0.8	158.0 $\pm$ 34.0	76.0 $\pm$ 2.6
Rat	0	92.0 $\pm$ 2.3	391.0 $\pm$ 31.0	434.0 $\pm$ 14.3	229.0 $\pm$ 10.4
	VLDL	175.0 $\pm$ 1.6	593.0 $\pm$ 18.2	874.0 $\pm$ 52.1	350.5 $\pm$ 8.5
	LDL	112.3 $\pm$ 1.5	508.0 $\pm$ 21.2	n.d.	n.d.

The experimental conditions were as described in the legend to Fig. 1, except that incubation was performed at 37° C; the ATP and Mg^{2+} concentrations were 0.2 mM and 5 mM respectively; the amount of membrane protein incubated was 2.2 μg (rat) and 1.5 μg (human). The concentration of lipoproteins was 200 μg protein ml $^{-1}$.

* Standard error of triplicate determinations.

n.d., Not determined.

gestion that VLDL transform the catalytic site of the cyclase into an activated state, in which it retains its susceptibility to stimulation by various effectors.

The mechanism involved in the observed activation by serum lipoproteins of the adenylate cyclase system in adipocyte plasma membranes remains indeterminate. The effect may, however, be dependent on some aspect of the native lipoprotein molecule, since isolated phospholipids (egg yolk lecithin and lysolecithin, added at concentrations equivalent to their content in native VLDL) lead to inhibition of the adenylate cyclase activity in fat cell membranes (preliminary observations). The observed activation of the adenylate cyclase system by VLDL might occur via an intermediate coupling step, involving lipoprotein binding sites on the adipocyte plasma membrane. Thus it seems likely that the apoprotein would be directly concerned, as has been demonstrated in the activation of the erythrocyte membrane Mg^{2+} -ATPase by VLDL (ref. 7). Alternatively, the activation of plasma membrane adenylate cyclase might occur by virtue of a physicochemical interaction between the serum lipoprotein particles themselves and plasma membranes. Thus, evidence has been provided for the exchange and transfer of cholesterol and of phospholipids between serum lipoproteins and a variety of cell membranes (for a review see ref. 18). Such processes, which may occur *in vivo*, may maintain or modify membrane fluidity and are thus implicated in the modulation of the immediate environment of the cyclase system; several lines of evidence indicate that the catalytic activity¹⁹⁻²¹ and hormone responsiveness^{20,22-25} of the adenylate cyclase system are strongly dependent on the nature of its surrounding lipids.

Papahadjopoulos²⁶ has suggested that serum β -lipoproteins may regulate the activity of several key membrane-bound enzymes, such as the ($Na^+ + K^+$) ATPase and adenylate cyclase. This hypothesis is consistent with the present observations which indicate that serum lipoproteins may stabilise or modify the adenylate cyclase system of fat cell membranes.

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Interaction of a fluorescent probe with acetylcholine-activated synaptic membrane

GRÜNHAGEN and Changeux have shown^{1,2} that *Torpedo* membrane fragments stained with the antimalarial, quinacrine, show a rapid increase in fluorescence after mixing with cholinergic agonists (see ref. 3). These authors suggest that the fluorescence intensity of quinacrine associated with cholinergic receptors increases during the transition of the receptor from its resting to its active state. We report here an electrophysiological analysis of the kinetic behaviour of the postsynaptic membrane of intact vertebrate muscle fibres treated with quinacrine. The kinetic data from the fluorescence and electrophysiological experiments show remarkable parallels, and we suggest that both experimental procedures reveal the binding of quinacrine to the activated receptor.

The experiments were carried out at room temperature (20-23 °C) on frog endplates using the voltage jump relaxation technique⁴⁻⁷. An endplate was voltage-clamped using a conventional two-electrode arrangement. The endplate current induced by iontophoretic or bath application of acetylcholine (ACh) or carbachol was measured either using extracellular focal recording⁴, or as the total current after subtracting current flowing in the absence of agonist⁵. The first set of experiments was performed using focal current recording and iontophoretic ACh application. The focal recording method yields very small currents recorded during voltage steps in the absence of agonist (upper traces in Fig. 1a, b, c). During ACh action but before treatment with quinacrine, control relaxations were obtained by stepping the membrane potential to a new level and observing the resulting changes in agonist-induced current (Fig. 1a). Following a step hyperpolarisation (marked by a downward arrow) the agonist-induced current jumps very rapidly from its previous steady level to the new level expected from the change in driving force on those channels that are already open just before the jump. The current then increases exponentially to a new steady level, as the ACh-evoked conductance increases from the equilibrium level appropriate to the original holding potential to the level appropriate to the potential during the jump. This conductance increase reflects opening of additional endplate channels. Over the range of agonist concentrations used in the present study, the normal open-close kinetics of the channel behave as if a single molecular inter-conversion takes place



The forward rate constant is increased by increasing the agonist concentration^{6,8} and the back rate constant is decreased by hyperpolarising the membrane⁹.

In the presence of 0.5 μ M quinacrine, the time course of the fast

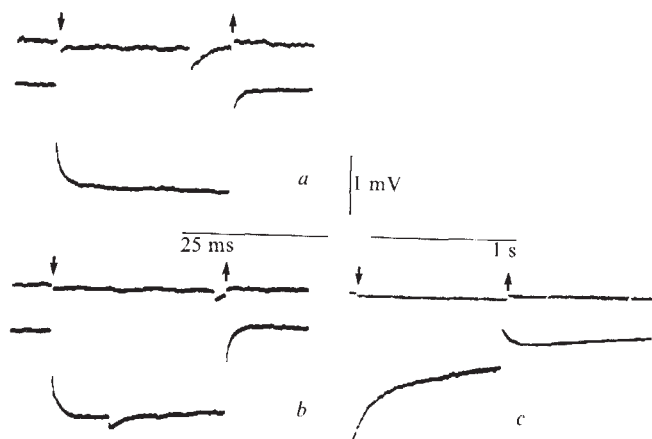


Fig. 1 Effect of quinacrine ($0.5 \mu\text{M}$) on relaxations induced by rectangular voltage jumps (from -60 mV to -100 mV at the downward arrow, and back to -60 mV at the upward arrow) during the action of iontophoretically applied ACh at a voltage-clamped sartorius endplate. Membrane current was recorded as the potential difference between a Ringer-filled fire-polished micropipette placed on the nerve terminal and a similar reference micropipette nearby. Downward deflections represent inward membrane currents. In each pair of traces, the upper trace shows the negligible leakage current during voltage jumps in the absence of ACh. The lower traces show responses to jumps during ACh iontophoretic applications (the absolute vertical shift between the upper and lower traces does not indicate the absolute value of the ACh-induced current at -60 mV). *a*, Control in the absence of quinacrine; *b*, and *c*, in the presence of $0.5 \mu\text{M}$ quinacrine. The same agonist dose was applied in *a*, *b* and *c*. A short jump was applied in *a* and *b* (25-ms time calibration), and a long jump in *c* (1-s time calibration). The extra ACh current which normally develops during the step was greatly decreased in the presence of quinacrine. In *c*, an additional slow inactivation process reflecting desensitisation shows up as an inclined base line. Miniature endplate currents are visible in the left-hand traces.

increase in conductance following a step from -60 mV to -100 mV was little changed (Fig. *ab*). The initial relaxation, however, was now followed by a slow decrease in current (Fig. *1b*), and if the hyperpolarisation was maintained, the extra agonist-induced current fell back to a low level (Fig. *1c*). Inactivation of agonist-induced current following a step hyperpolarisation was not seen in the absence of quinacrine in the conditions of the present experiments. On stepping back from -100 mV to -60 mV (upward arrows) there occurred, successively, a very fast ohmic decrease in current, a fast relaxational decrease in current and finally a slow increase in current (Fig. *1b* and *c*). When applying ACh iontophoretically, the slow relaxation was not always exponential, probably because of local variations in agonist concentration or concurrent desensitisation.

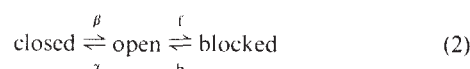
The slow relaxation was then studied in more detail using bath agonist application, total current recording and a fixed quinacrine concentration of $1 \mu\text{M}$. In these conditions (Fig. 2), the slow relaxation, measured after subtracting currents flowing during the step in the absence of agonist, was exponential. Its time constant became smaller as the concentration of agonist (ACh or carbachol) was increased (Figs 2 and 3). The times for half decay of the slow relaxation for -60 mV to -140 mV jumps are shown as a function of agonist concentration in Fig. 3. Individual half-decay times ranged from 2 to 0.04 s . Higher concentrations of carbachol than of ACh were needed to give equal half-decay times.

Our results show that even at low concentrations, quinacrine profoundly modifies the normal kinetic behaviour of the cholinergic membrane, and that in steady-state conditions, it gives rise to a blocking effect more pronounced at more hyperpolarised potentials. This effect of quinacrine at the endplate strikingly resembles the action of procaine on *Aplysia* neurones¹⁰, and may thus reflect the local anaesthetic action of quinacrine postulated by Grünhagen and Changeux¹¹.

The kinetic data reported here, obtained electrophysiologically, and those revealed by fluorescence^{2,3}, display resemblances when

using the same two agonists, in spite of differences in the techniques and preparations used. Since in the present experiments the slow process corresponds to a decrease in conductance, it is possible that the fluorescence increase seen when adding the agonist to microsome suspensions and which has a similar slow time course, does not monitor directly the activation of the receptor. Instead, we propose that quinacrine, like other local anaesthetics^{12,13}, blocks activated receptor-channel complexes. One interpretation of the voltage dependence of the quinacrine effect would thus be voltage sensitivity of the partitioning of cationic quinacrine between the external membrane surface and a blocking site within the channel¹⁴.

In the presence of quinacrine, the scheme describing endplate channel kinetics can be modified to



This scheme predicts two relaxation processes, as observed. It may be shown that if the two relaxation times are well separated, scheme 2 leads to the following expressions for the fast (τ_f) and slow (τ_s) relaxation time constants

$$1/\tau_f = \alpha + \beta + f + b$$

$$1/\tau_s = \frac{\beta f + \alpha b + \beta b}{\alpha + \beta + f + b}$$

In our experiments, the time constant of the control relaxation, $1/(\beta + \alpha)$, was very similar to that of the fast relaxation in the presence of quinacrine. This implies $(\beta + \alpha) \gg (f + b)$. With this condition the equation for τ_s simplifies to $1/\tau_s = f\beta/(\beta + \alpha) + b$.

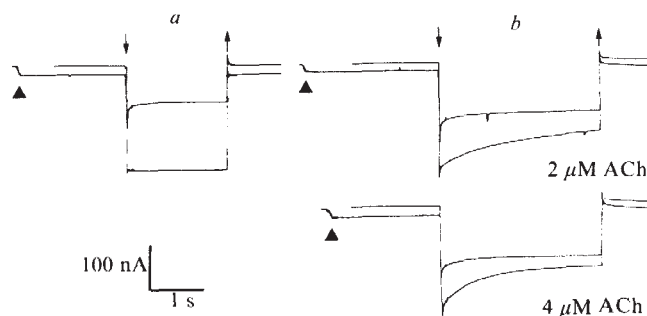


Fig. 2 Slow quinacrine-induced relaxations during bath application of ACh to a voltage-clamped frog muscle endplate. ACh responses to fast agonist superfusion had times to peak less than 10 s . In each pair of traces, the upper trace shows the total clamp current recorded in the absence of ACh before, during and after rectangular hyperpolarising voltage jumps (from -60 mV to -140 mV , downward arrow, and back to -60 mV , upward arrow). Apart from the initial capacity transients, the passive currents are almost constant during the jumps. The lower traces in each pair show inward currents recorded during ACh action at 1-s time calibration, except for the beginning of each trace which shows the onset of the response to ACh perfusion ($\blacktriangle$) at a tenfold slower time base. The difference between the upper and lower traces in each block represents the ACh-induced endplate current. In the absence of quinacrine (*a*) the additional ACh-induced ($2 \mu\text{M}$) current flowing during the step is well maintained. In the presence of $1 \mu\text{M}$ quinacrine (*b*), the ACh current is not well maintained and shows a progressive decrease. This slow relaxation is clearly faster for $4 \mu\text{M}$ ACh than for $2 \mu\text{M}$. Note that the current at the holding potential evoked by $2 \mu\text{M}$ ACh is only slightly depressed by quinacrine. The fast relaxations in the absence and the presence of quinacrine cannot be resolved with total current recordings. In all bath application experiments using ACh, endplate cholinesterase was inactivated by a 90-min treatment with methyl, *O*-ethyl, 5-ethylidipropylamine phosphorothiolate at $0.5 \mu\text{M}$, followed by extensive washing. After the treatment, $25\text{--}30 \mu\text{M}$ carbachol was needed to match the response to $1 \mu\text{M}$ ACh; this was used as an indication of cholinesterase block. Cholinesterase was blocked so that the ACh concentration in the synaptic cleft would equal the bath concentration.

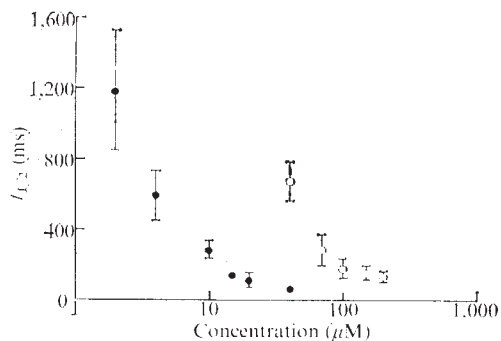


Fig. 3 Mean half-decay times (evaluated from semilog plots of relaxing currents) of slow quinacrine-induced relaxations at -140 mV membrane potential. The quinacrine concentration was $1 \mu\text{M}$, and the agonists were applied in the bath. The bars show ± 2 s.e.m., where this exceeds the symbol size. Experiments were from 13 *Rana pipiens* fibres. The agonist concentration (abscissa) is on a log scale. The data were plotted in this manner to facilitate comparison with Fig. 2 of ref. 2. ●, Acetylcholine; ○, carbachol.

Noise and relaxation experiments^{6,8} in the absence of quinacrine, which provide a direct measure of $(\beta + \alpha)$, show that β increases monotonically as the agonist concentration is increased, in a range comparable with that used here. Our model therefore predicts the monotonic decrease in τ , as the agonist concentration is increased (Fig. 3). The observation that for high agonist concentrations τ seems to reach a limiting value which is larger for carbachol than for ACh (Fig. 3 and Fig. 2 of ref. 3) suggests that carbachol cannot open all the endplate channels, even when acting at high concentration. In this model, the electrophysiological and fluorescence data are to be interpreted in slightly different terms, since the first would reflect the number of conducting channels remaining after quinacrine treatment, whereas intensity of fluorescence would directly monitor the amount of quinacrine bound to the receptor-channel complex.

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Role of intracellular calcium in the transient outward current of calf Purkinje fibres

CALCIUM-activated currents are known in red blood cells¹, invertebrate neurones², vertebrate spinal motoneurones³ and skate electroreceptors⁴, and it has been suggested that intracellular calcium increases outward currents in cardiac muscle^{5–7}. The possibility of calcium-activated currents in heart and other muscle^{8,9} is interesting because these cells have intracellular stores that release calcium. Some of the apparent complexity of membrane currents in muscle might be explained as a manifestation of excitation-contraction coupling. If internal calcium (Ca_i) en-

hanced a surface membrane conductance, one might expect a phasic current which reflected the increase and decrease in Ca_i during contraction. No such current has been identified in heart. We report evidence here that intracellular calcium controls the transient outward current which underlies the early repolarisation in cardiac Purkinje fibres^{10,11}. Thus the transient outward current is inhibited by blockers of calcium current, varies directly with the external calcium concentration and correlates with twitch amplitude in conditions which vary the amount of calcium released.

Short Purkinje fibre preparations from calf hearts were voltage-clamped by a conventional two-microelectrode method. In some experiments, contractile force was measured with a piezoelectric force transducer (Endevco 87102). Interference from the excitatory sodium current was prevented by blocking sodium channels with tetrodotoxin ($20 \mu\text{M}$) or inactivating them with a depolarised holding potential (~ -40 mV). The transient outward current is referred to as I_{qr} because the kinetic variables q and r have been used previously to describe its activation and inactivation^{11,12}.

In Fig. 1a, trace 1 shows a record of membrane current during a clamp depolarisation in the range of the action potential plateau. The outward transient current (I_{qr}) is dominant for the first 150 ms after the step depolarisation and obscures the slow inward calcium current (I_{si}). I_{si} turns on within a few ms after the depolarisation and then inactivates slowly¹³; the inactivation is largely responsible for the slow current change during the last 300 ms of the pulse. Efforts at quantitative analysis of I_{si} in Purkinje fibres have been hampered by such overlap with I_{qr} (refs 14, 15). We tried to determine I_{si} without interference from I_{qr} by a pharmacological dissection involving D600, a blocker of calcium currents¹⁶. But D600 inhibited the outward transient as well as I_{si} . In preparations showing large I_{qr} (Fig. 1a) the early current in the absence of D600 (1) was more outward than the current in its presence (2). Thus, the 'D600-sensitive current' (1–2, Fig. 1b) displayed a region of net outward current. We attribute the outward region to I_{qr} (shaded area) in combination with I_{si} . The inward spike in Fig. 1b is explained as a rapid activation of I_{si} preceding the activation of I_{qr} . Similar net current waveforms have been observed in crustacean muscle and have been analysed as a transient outward current superimposed on an inward calcium current^{9,17}.

The consistent reduction of I_{qr} obtained with D600 invalidates the use of this drug to determine I_{si} . But the results also suggested that I_{qr} is dependent on intracellular calcium. On this view, the reduction of I_{qr} would be accounted for as a natural consequence of the inhibition of calcium entry. We explored this hypothesis using procedures believed to modify calcium influx. Figure 1c shows that manganese ion, another widely-used blocker of calcium currents^{13,14}, resembles D600 in the ability to reduce I_{qr} . Figure 1d shows the effects of increasing the extracellular calcium concentration from 1.8 to 5.4 mM. Increased slow inward current is evident from the augmented early spike (arrows) and the downward displacement of current during the last 400 ms of the clamp pulse. The increase in external calcium (Ca_0) also produced a clear enhancement of I_{qr} which was fully reversible (record not shown). An increase of Ca_0 from 5.4 to 9.0 mM caused a further increase in I_{qr} .

Another approach to the possible involvement of intracellular calcium relied on contractile force to report changes in myoplasmic calcium activity. We took the amplitude of the twitch as a rough index of the calcium transient and compared it with the surge of I_{qr} during depolarising clamp pulses. The size of the calcium transient was varied by manipulating the previous history of membrane potential¹⁸. Figure 2a shows a typical voltage clamp protocol consisting of two identical depolarising pulses separated by a variable interval. The first pulse followed a 5-s quiescent period and produced a large transient outward current and a sizeable twitch contraction. The second pulse was applied after a 1.5-s repolarisation and evoked a smaller I_{qr} and smaller twitch. Earlier experiments of this type have shown that the level and duration of the repolarisation strongly influence the restitution of the second contraction¹⁸ and the recovery of the outward transient current¹¹. Here the question is whether these recovery processes share the same time and voltage dependence. Because this

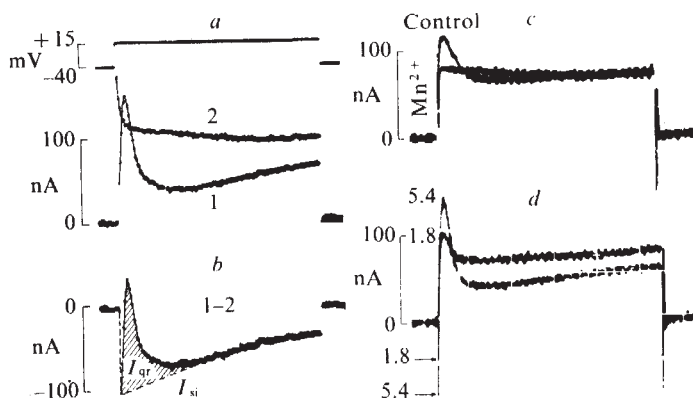


Fig. 1 Effects of D600, manganese and calcium on the transient outward current (I_{qr}). *a*, Membrane current in the absence of drug (1) and after exposure to D600 (5×10^{-6} g ml $^{-1}$) for 30 min (2). The current records accompanied a rectangular membrane depolarisation from -40 to $+15$ mV for 500 ms (upper trace). Signals were recorded by a digital computer, sampling once every 3 ms. The smooth line was drawn by eye for clarity; preparation S22-1. *b*, 'D600-sensitive current', obtained by subtracting trace (2) from trace (1). Shaded area is estimate of I_{qr} , dashed line estimate of I_{si} . *c*, Membrane current in a control run and after exposure to 3 mM MnCl $_2$ for 9 min. The currents were evoked by a 500-ms clamp pulse from -38 to $+13$ mV. *d*, Membrane current in 1.8 mM Ca and 10 min after increasing the external calcium concentration to 5.4 mM. The arrows indicate the peak inward spike of current, an approximate measure of peak I_{si} . Currents were in response to a 520-ms clamp pulse from -39 to $+13$ mV. *c* and *d*, Preparation R33. 2. The composition of the Tyrode solution used in these experiments was 150 mM NaCl, 4 mM KCl, 10 mM Tris-HCl buffer, pH 7.4, 0.5 mM MgCl $_2$, 5.4 mM CaCl $_2$ and 3 mM glucose.

and I_{si} were particularly distinct. Clearly the amplitudes of I_{qr} , I_{si} and twitch force were each enhanced by a second pulse relative to their size during the first pulse.

These kinetic correlations between I_{qr} and twitch force, taken together with the actions of D600, manganese and extracellular calcium, strongly suggest that the transient outward current is controlled by intracellular calcium activity. The myoplasmic calcium could be supplied by release from intracellular stores or entry across the surface membrane. Contractile force provides a convenient and sensitive bioassay for the size of the intracellular calcium transient. This approach has drawbacks, however, since the peak of the twitch must lag behind the peak of calcium activity near the surface membrane because of diffusional, chemical and mechanical delays. It is possible that the waveform of I_{qr} is a more faithful representation of the time course of calcium activity than the externally measured force. Direct measurements of the calcium transient may soon be possible using optical indicators^{21,22}. Such experiments would provide another test for the present hypothesis and might determine whether intracellular calcium governs the time course of I_{qr} or if it merely acts as a trigger for a phasic conductance change.

Our results suggest that some of the time and voltage dependence of the transient outward current arises from the same mechanisms which underly activation and inactivation of contraction. The ionic nature of the outward current remains unsettled. Chloride was originally thought to carry I_{qr} because the outward transient was reduced by replacing chloride with larger anions^{10,11}. But Kenyon and Gibbons²³ have shown that the

comparison depends on a fairly accurate determination of I_{qr} , we reduced interference from I_{si} by choosing preparations with relatively large I_{qr} , by using strong depolarisations to increase the driving force for outward current and by estimating I_{qr} with a graphical procedure (Fig. 2*a*) which made some allowance for I_{si} .

Figure 2*b* shows the influence of potential level on the recovery of the twitch and of I_{qr} . The results were obtained with a 1.5-s repolarisation interval and were normalised relative to the responses evoked by the first pulse. The contraction and the transient outward current show nearly identical dependence on membrane potential. Similar correspondence was observed in five out of six preparations. Figure 2*c* shows the recovery of the twitch and I_{qr} as the repolarisation interval increased progressively. Both signals show a similar time course. Temporal correlation was observed in six out of seven experiments of this kind, and was found at -70 mV as well as near -40 mV. The occasional lack of correlation might be explained by exceptionally severe contamination by I_{si} .

Although parallelism between I_{qr} and contractile force is clear and rather consistent, the agreement could be considered fortuitous. A purely coincidental explanation is not supported, however, by experiments in which the recovery time course was manipulated drastically. Cardiac glycosides and aglycones were useful for this because they modify the time course of contractile restitution as well as increasing the strength of contraction¹⁹. Figure 3 shows the influence of strophanthidin on the recovery of the twitch and of I_{qr} . In the absence of the drug, both transients showed a monotonic recovery with increasing repolarisation interval as in Fig. 2*c*. After exposure to 1 μ M strophanthidin, contractile restitution and recovery of I_{qr} both became oscillatory, with the same period and phase. The discrepancy in the height of the oscillatory peaks at 0.5 s could be explained if I_{qr} were more seriously underestimated at this time. Contamination from I_{si} may have been more severe at the peak because other experiments have shown that strophanthidin evokes a clear oscillation in the repriming of I_{si} (ref. 20). The inset in Fig. 3 illustrates another experiment using strophanthidin in which the time courses of I_{qr}

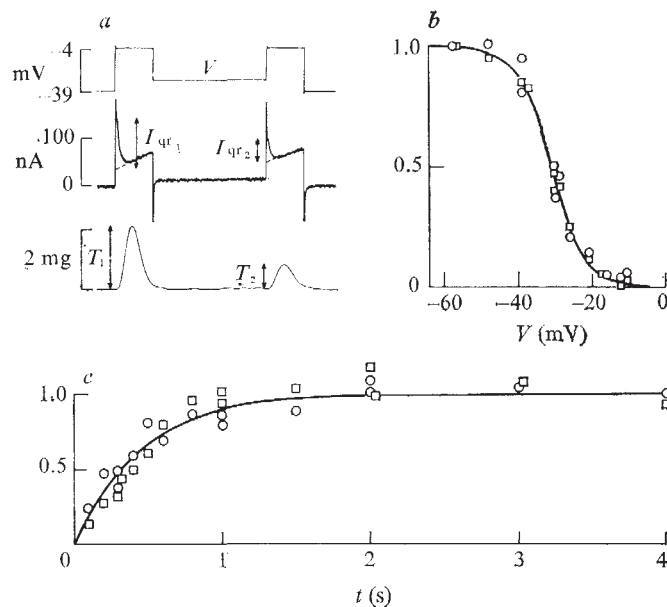


Fig. 2 *a*, Experimental records illustrating the protocol for obtaining the curves in (b) and (c). The traces (records from a Brush 440 pen recorder) show, from top to bottom, membrane voltage, membrane current and contractile force. The magnitude of I_{qr} was measured as the difference between the peak of the outward transient and a linear extrapolation of the late current to the time of peak I_{qr} (dashed line). This method will underestimate I_{qr} because it underestimates I_{si} . To correct for small progressive changes during the run, currents and tension during the second pulse were normalised by the response to the first pulse. *b*, Relationship between repriming potential (V) and the inactivation of I_{qr} ($\square$) and tension ($\circ$). The normalised I_{qr} and tension at a given repriming potential were divided by the normalised values for $V = -57$ mV to yield the inactivation curve. The continuous line was drawn according to $r = [1 + \exp((V + 31)/4)]^{-1}$, where r is a Hodgkin-Huxley type variable with values between 0 and 1. *c*, Comparison of the recovery from inactivation of I_{qr} and tension. Two depolarising voltage-clamp pulses were separated by repolarisation to the holding potential for a variable interval (t). Normalised I_{qr} and tension were plotted as a function of t . Symbols are as in (b). The continuous line is an exponential with a time constant of 450 ms. Voltage-clamp pulses in (a), (b) and (c) depolarised the membrane to -4 mV for 500 ms from a holding potential of -39 mV; preparation R25-2.

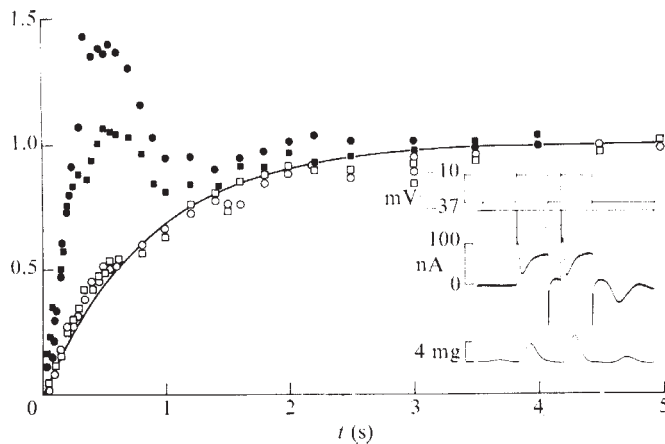


Fig. 3 Alteration of the repriming time courses of I_{qr} and tension by strophanthidin. In the absence of strophanthidin the repriming time courses of I_{qr} ($\square$) and tension ($\circ$) are very similar. The continuous line is an exponential with a time constant of 880 ms. The closed symbols were obtained 15 min after addition of $1 \mu\text{M}$ strophanthidin. Both repriming curves then show distinct oscillations. Repriming curves obtained with 500-ms clamp pulses from -40 mV to -10 mV . Preparation R27-2. The inset shows a chart recording from a less complete experiment in the presence of $1 \mu\text{M}$ strophanthidin: preparation R26-7.

substitute anions act by reducing extracellular calcium activity. Their results are consistent with the sensitivity of I_{qr} to deliberate variations in Ca_0 (Fig. 1d). Reports of calcium-activated potassium currents in other tissues raise the possibility that potassium ions help carry I_{qr} . Whatever the ionic basis of I_{qr} , its apparent dependence on intracellular calcium raises fundamental difficulties for analysis of the slow inward current in Purkinje fibres. A drug which interacts specifically with Ca_i channels will still have indirect effects on I_{qr} . Thus proper pharmacological dissection of Purkinje fibre plateau currents will require a method for selectively abolishing I_{qr} .

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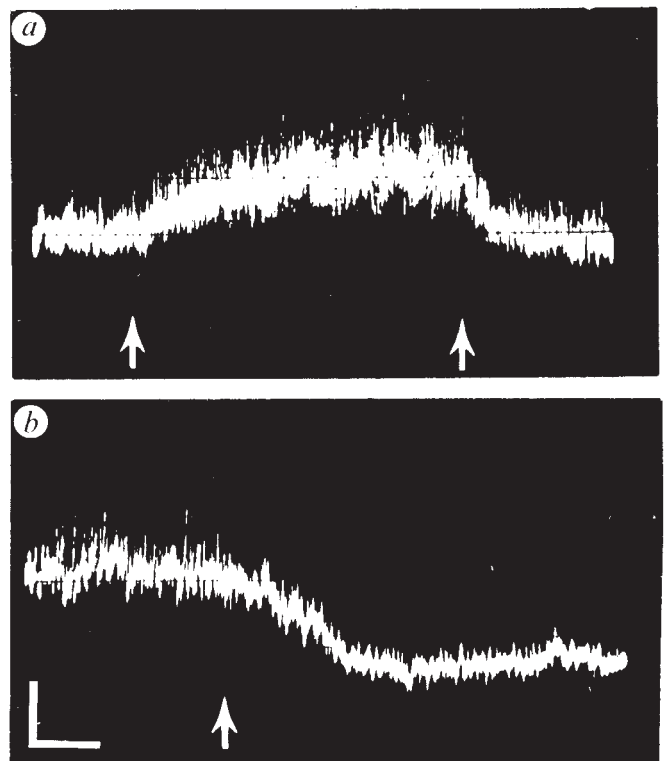
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Voltage noise from hair cells during mechanical stimulation

THE generator potential of mechanoreceptor hair cells is thought to arise from a change in conductance of the receptor membrane^{1–4}. A mechanical force in the environment acts on hairs to cause the generator potential. The conductance change associated with the generator potential may be composed of many elementary conductances whose frequency or number vary with mechanical stimulation. From this hypothesis, one would expect to observe random fluctuations, or noise, associated with the generator potential. We report here the presence of such fluctuations in an invertebrate statocyst; the analysis of the fluctuations gives an estimate of the size of the underlying events associated with the generator potential during mechanical stimulation.

Hair cells of the invertebrate statocyst are presumably stimulated by statoconia. Increased interaction between statoconia and the cilia depolarises the hair cells; decreased interaction hyperpolarises them. The statoconia, however, are in constant motion. Random movement of these particles should cause a baseline noise^{1–3}. Figure 1 shows intracellular recordings from a hyperpolarised hair cell body of *Hermisenda crassicornis*. The cells were hyperpolarised by injecting current through the recording electrode to suppress spontaneous firing which normally occurs near rest at -55 mV . The preparation and the rotational method of physiological stimulation have been described elsewhere³. Hair cell somata were passively invaded by action potentials arising in the axon $80 \mu\text{m}$ from the soma, and received synaptic input at axonal branches $100 \mu\text{m}$ from the soma. The generator potential and voltage noise can be studied in the absence of impulses and synaptic interactions by cutting the distal axon (cut N⁵). Fig. 1a shows a depolarising response from a hair cell (cut N) in front of the force vector. In the absence of rotation, the variance in the voltage noise was $9.3 \times 10^{-8} \text{ V}^2$. A rotational velocity of 60 r.p.m.

Fig. 1 Intracellular recordings (0–313 Hz) from statocyst hair cells. The vertical bar represents 5 mV, the horizontal bar 5 s. *a*, A hair cell in front of the force vector with cut axon (cut N) to remove synaptic input. Rotation begins and ends at the arrows, and produces a maximum steady value of 0.8 g midway during the response. The holding potential is -100 mV . *b*, A hair cell with intact axon behind the force vector. Rotation begins at the arrow and produces a maximum steady value of 1.4 g. The holding potential is -80 mV .



for a cell located 19 cm from the centre of rotation, produced an average depolarisation of 5.5 mV and an increase in the variance to $29.3 \times 10^{-8} V^2$. Figure 1b shows the opposite effect from a cell behind the force vector, the axon of which was left intact. The noise variance decreased from 12.0×10^{-8} to $2.1 \times 10^{-8} V^2$ as a result of a rotational velocity of 75 r.p.m. The mean hyperpolarisation was 9 mV.

The variance of the microelectrode and amplifier noise, measured in the external bath, was less than $10^{-8} V^2$. The noise increased as the microtip entered the statocyst. Hyperpolarising the membrane with current further increased the noise (Fig. 2). Microelectrode noise measured in the external bath increased negligibly over the current range used. The voltage was held between -80 and -100 mV in the rotation experiments. In this range, the 'background noise', that is, membrane noise plus microelectrode and amplifier noise, varied between 2×10^{-8} and $10 \times 10^{-8} V^2$. Changes were then studied as a function of the physiological stimulus.

Autocorrelation functions of the noise are shown in Fig. 3. At -80 mV and zero rotational velocity, the correlation function is approximated by a single exponential with time constant 74 ms. The 7-mV hyperpolarisation caused by rotation reduced the noise and increased the time constant to 168 ms. An increase in the speed of rotation, increased the hyperpolarisation to -9 mV and reduced the noise, with little further effect on the time constant. The noise level at -9 mV approached that caused by microelectrode and amplifier noise. A cell with similar background noise is shown for the depolarising response. Depolarisation to 16 mV increased the noise variance with no appreciable change in time constant. The smallest time constant observed for a depolarising response was 62 ms. The largest time constant observed for a hyperpolarising response was 178 ms.

The results of many experiments are summarised in Fig. 4 in which the change in noise variance is compared with the mean change in membrane potential, without regard to sign. Hyperpolarising and depolarising responses are presented for both intact hair cells and from hair cells with cut axons (cut N). The change in variance was roughly proportional to the change in the mean. A solid line, approximately determined by the data points, has a

Fig. 2 Intracellular recordings from statocyst hair cells, hyperpolarised from their resting value by current injection. The average resting potential is about -55 mV. One very noisy cell (upper dashed line) and one very quiet cell (lower dashed line) are shown. The solid line is drawn through pooled data from many cells.

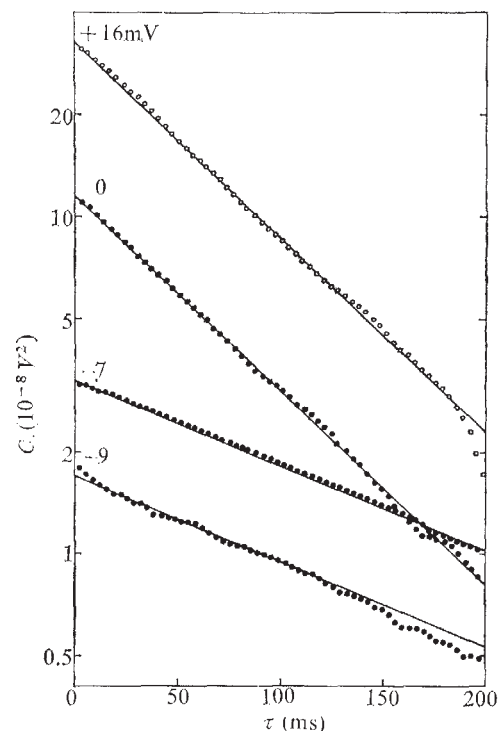
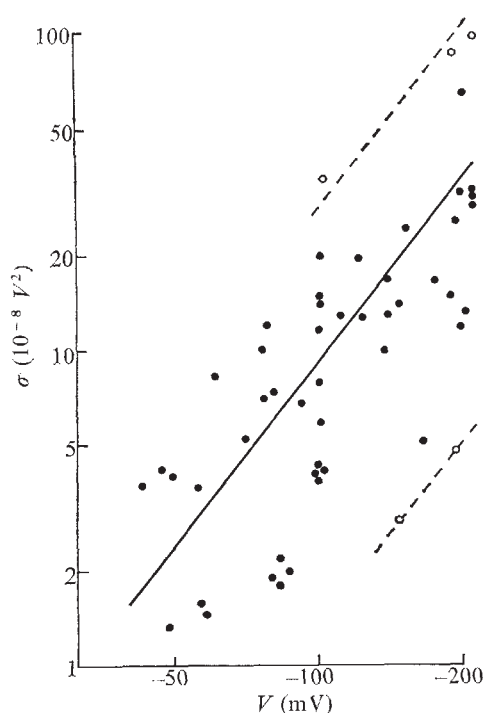


Fig. 3 Autocorrelation functions of the noise in various conditions of rotation. The sample length was 27.31 s during steady rotation. The data were recorded on FM tape at 3.75 i.p.s. (0-625 Hz) after passing the signal through an RC high pass filter with a 2.2 s time constant. Processing was carried out off-line with a d.c.-coupled HP 3721A correlator after passing the signal through a RC high pass filter with an 8 s time constant. ●, Noise from a hair cell behind the force vector, at zero rotation and two levels of rotation which produce steady hyperpolarisations of 7 mV at 1.2 g and 9 mV at 1.4 g. ○, Depolarising response of 16 mV at 1.2 g from a cell with approximately the same zero rotation background noise. The lines represent exponentials with time constants 74 ms (upper) and 168 ms (lower). C is the value of the autocorrelation for relative time displacement τ .

slope of $12 \mu V$. This slope may be taken as the order of magnitude of the elementary voltage event associated with mechanotransduction. The dashed line in Fig. 4 represents the expected distortion of the variance-to-mean relationship produced by the summation of elementary increases in conductance. The soma membrane resistance with rest potential E is taken in parallel with the receptor membrane resistance with reversal potential e . The correction factor to $\Delta\sigma^2$ is f^4 and to ΔV is f , where $f = V_0/(V_0 - \Delta V)$ and $V_0 = e - E$ is the maximum possible ΔV . This analysis is similar to that applied to acetylcholine-induced noise at the neuromuscular junction⁶ and to changes in noise induced by light in bipolar cells and photoreceptors⁷.

Two experiments in Fig. 4 are from an abnormal *Hermisenda* statocyst which has only one or two statoconia instead of several hundred. Such a statocyst, which may be a mutant form, offers the possibility of studying the elementary statoconium-hair cell interaction more directly.

Are the changes in noise produced by rotation caused by the mechanotransduction or by changes in membrane potential? A change in membrane potential by current injection has the opposite effect to a change caused by physiological stimulation. For example, a hyperpolarisation caused by current increased the noise (Fig. 2), but a hyperpolarisation caused by rotation decreased the noise (Figs 1 and 4). The converse was true for the depolarising direction. We conclude that the changes in hair cell noise analysed here are caused by mechanotransduction.

Since the change in variance is roughly proportional to the mean, the potential changes due to mechanotransduction can be considered as the sum of random events according to a simple shot model. The model is discussed fully by Katz and Miledi⁸. The basic relationship for a random sum of exponential events, each of height a is: $a = 2\Delta\sigma^2/\Delta V = 24 \mu V$. The spread of data in Fig. 4 is

due to a variation in the time constant with different levels of rotation (Fig. 3) and to the subtraction procedure.

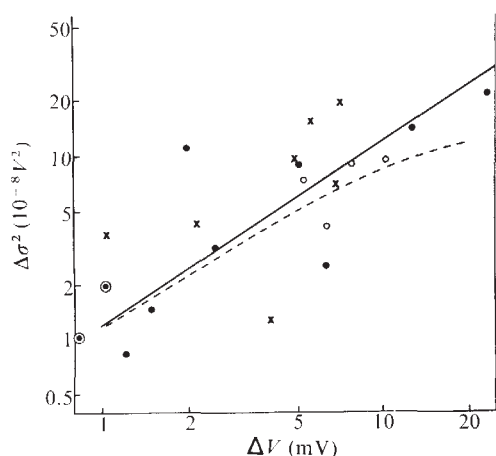
The largest time constant previously observed by the displacement of voltage with current pulses was 45 ms^{-1} . The relaxation times shown in Fig. 3 may be caused by transduction or by an induced change in input resistance. The input resistance could be expected to decrease for the depolarising response and increase for the hyperpolarising response, although we have no direct evidence that this occurs. In either case, a random event with a single relaxation time for a particular stimulus describes the effect shown in Fig. 4. The input resistance of the hair cells at rest is $80\text{--}100 \text{ M}\Omega$. If the amplitude of an individual event is taken as $24 \mu\text{V}$, one calculates an elementary current of approximately 0.3 pA for each event. It was shown recently that hair cell generator potentials and voltage noise are dependent equally on sensory stimuli, membrane potential, external sodium concentration, and treatment with the anaesthetic, chloral hydrate (D.L.A., Y. Grossman and E. Heldmann, unpublished).

These observations support the hypothesis that the voltage noise reported here involves the same mechanism that underlies the generator potential. The generator potential arises from a conductance increase with a reversal potential of about 30 mV positive with respect to rest¹. From an average holding potential of -90 mV , we assume a driving force of 60 mV . The elementary conductance change associated with each event is therefore about 5 pS . This is the order of conductance found for individual ionic channels⁸. Although there is considerable uncertainty in the reversal potential, even if 30 mV positive with respect to zero is assumed, the estimate for the elementary conductance change is merely halved.

Our observations show that the changes in hair cell membrane potential do not account for the changes in noise produced by a physiological stimulus. The changes in noise produced by rotation are primarily caused by the transduction process itself, indicating a common origin for voltage noise caused by rotation and by the hair cell generator potential. We conclude that the generator potential is the result of a random sum of elementary events which have an average effect (the generator potential) and a fluctuating component (the voltage noise). Local potential phenomenon previously reported by Alkon³ do not occur at the holding potentials used here, nor do they occur in hair cells with cut axons. Thus, such effects could not contribute to the noise studied here.

The number of hairs interacting with one statoconium is

Fig. 4 ¹ Change in noise variance ($\Delta\sigma^2$) against the change in mean membrane potential (ΔV) at different levels of rotation. $\times$, Hair cells with cut axons (cut N) showing a depolarising response. Hair cells with intact axons showing a depolarising ($\circ$) or hyperpolarising ($\bullet$) response. $\odot$, From an abnormal statoconium with one or two statoconia. Eleven different cells are represented in this figure. The solid line represents $\Delta\sigma^2/\Delta V = 12 \mu\text{V}$. The dashed curve represents the expected nonlinear effect of increased conductance on the depolarising response assuming a maximum ΔV of 90 mV . The variance was taken as the first point of autocorrelation functions calculated from 13.65 or 27.31 s of data.



estimated to be between 1 and 20, based on scanning electron micrographs (D.L.A., Y. Grossman and E. Heldmann, unpublished). Discrete depolarising waves observed in the voltage noise near rest³ probably reflect the interaction of a statoconium with one or more cilia. These depolarising waves range from $1\text{--}10 \text{ mV}$. If the lower limit of 1 mV is taken to approximate the interaction of a single statoconium with a single hair, 40 elementary events, each causing a 5-pS increase of conductance, would be necessary. Noise analysis offers the possibility of relating molecular membrane phenomena, which are not directly observable, to the mechanism of the generator potential of a sensory receptor.

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Stimulation of the sympathetic nervous system during sucrose feeding

THE activity of the sympathetic nervous system in the rat is markedly reduced by 2 d of fasting, and this suppression is reversed by feeding¹. Studies of urinary catecholamine excretion in fasting rats have corroborated these findings and in addition have shown that urinary adrenaline and noradrenaline (NA) excretion actually exceed baseline levels on the second and third days of refeeding². Since dietary intake in these previously fasted animals was greater than baseline during the refeeding period, the possibility arose that overfeeding might be associated with increased sympathetic activity and led to the present investigation. Work by others has provided evidence that supplementation of a standard rat chow diet with sucrose-containing drinking solutions increases total caloric intake of the rat by approximately 20%³. Thus the experimental design for voluntary overfeeding in the rat is based on the addition of sucrose-containing drinking solutions to the animals' *ad libitum* access to rat chow and water during the experimental period. In this report we describe increased sympathetic activity in the hearts of overfed animals after 3 d of sucrose supplementation. Such an increase in sympathetic activity may contribute to the enhanced thermogenesis associated with overfeeding.

The use of NA turnover techniques in assessing the functional state of the unperturbed sympathetic nervous system permits the estimation of organ-specific sympathetic activity in each sympathetically innervated organ. NA, the sympathetic neurotransmitter, is synthesised and stored within the sympathetic nerve endings and is released in response to neural impulses. An active transport mechanism in the neuronal membrane recaptures a major fraction of the released NA. As the activities of both NA biosynthesis and NA re-uptake are coupled to the level of impulse traffic, the endogenous level of NA within the nerve terminals in a particular organ is relatively constant despite wide fluctuations in sympathetic activity. The kinetic measurements of NA turnover used here use these neuronal processes of NA biosynthesis and active transport to quantitate changes in the level of sympathetic activity in experimental conditions. The ³H-NA turnover

technique involves the intravenous (i.v.) administration of tracer doses of ^3H -NA. After uptake of the label into the sympathetic nerve endings and equilibration with intraneuronal stores, the rate of disappearance of the tracer provides an indication of sympathetic activity within a particular organ. Previous work with this technique has established its usefulness as a measure of sympathetic activity in various physiological and pathophysiological conditions⁴⁻⁶. A second method of assessing NA turnover involves inhibition of NA biosynthesis with α -methyl-*P*-tyrosine (α MPT), an inhibitor of tyrosine hydroxylase, the rate-limiting step in NA biosynthesis. In the presence of such inhibition endogenous NA levels cannot be maintained and the rate of fall of endogenous NA reflects NA turnover. Intraneuronal metabolism of NA contributes to a limited extent to these measurements of NA turnover, but is not altered by the experimental conditions described here.

Female Sprague-Dawley rats (150–200 g) were used in these experiments. These animals were allowed free access to Purina rat chow and water except as noted in the experimental protocols. The effect of 3 d of sucrose feeding on NA turnover as measured by the rate of disappearance of ^3H -NA is shown in Fig. 1. In this experiment control (normal diet) and sucrose-fed animals received an i.v.

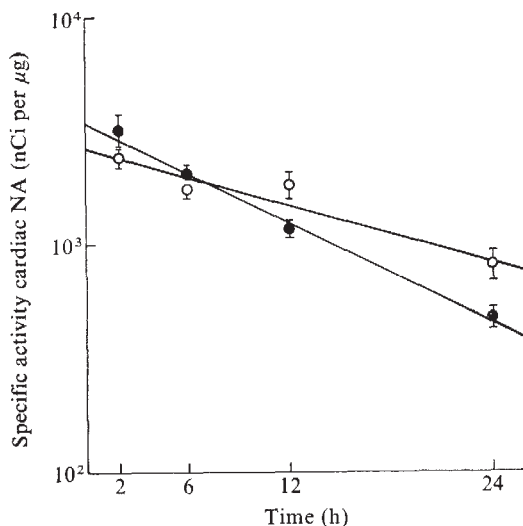


Fig. 1 ^3H -NA turnover in rat heart in control and sucrose-fed animals. Female Sprague-Dawley rats (150–200 g) were divided into two groups 3 d before the start of the NA turnover experiment. Control animals (○) remained on their *ad libitum* diet of rat chow and water; sucrose-fed animals (●) were provided access to an 8% sucrose solution in addition to their rat chow and water. At the beginning of the turnover, each animal received an i.v. injection of ^3H -NA (250 μCi per kg body weight; specific activity 24.4 Ci mmol⁻¹; New England Nuclear) by tail vein. At 2, 6, 12 and 24 h after injection, five to seven rats from each group were killed, their hearts removed and quickly frozen on dry ice. The organs were stored at -20°C until analysed within 2–3 d. After the organs were homogenised in ice-cold 0.4 M perchloric acid, NA was isolated chromatographically with alumina and the eluates analysed for ^3H -NA and NA content, as previously described⁶. Values were corrected for recovery (80–90%) as determined in each experiment. Specific activity of cardiac NA was determined for each time point, expressed as mean $\pm$ s.e.m. The line representing the decline in specific activity with time was calculated by the method of least squares. For both control and sucrose-fed groups, the significance of each slope is $P < 0.001$. For the control group the slope or fractional turnover rate is $4.80 \pm 0.16\%$ per h with a NA turnover rate of 21.4 ± 1.9 ng NA per heart per h (95% confidence interval). For the sucrose-fed group the fractional turnover rate is $8.49 \pm 0.13\%$ per h and the NA turnover rate 30.6 ± 2.3 ng NA per heart per h (95% confidence interval). The slopes are significantly different at the $P < 0.001$ level using the Student's *t* test. Sucrose intake averaged 5.5 g per rat per d for the experiment. For control, $t_1 = 14.5 \pm 0.5$ h; for sucrose-fed rats, $t_1 = 8.2 \pm 0.1$ h.

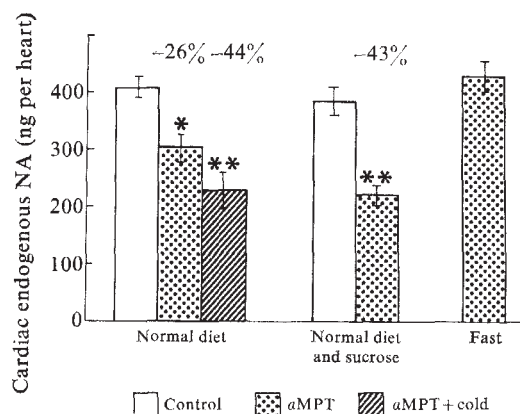


Fig. 2 Effect of inhibition of NA biosynthesis on endogenous NA levels in rat heart from control (normal diet), fasted and sucrose-fed animals. Animals were prepared as described in Fig. 1, except that in this experiment, a single group of animals was fasted for 48 h before the start of the experiment. Animals were injected i.p. with the methyl ester of α MPT (Sigma) dissolved in saline, 250 mg per kg at zero time and 125 mg per kg at 3.5 h; controls received the diluent alone in two injections. Animals were killed at 7 h, and the endogenous NA levels in heart determined. Open bars represent the saline-treated animals, the shaded bars the α MPT-treated animals, and the striped bar a single group of α MPT-treated animals that was placed in cages in a cold (4°C) room during the course of the experiment. Each group contained eight or nine animals. * Represents $P < 0.005$ and ** represents $P < 0.001$, when compared with the corresponding diluent control. The endogenous NA content in α MPT-treated animals is significantly less ($P < 0.02$) in those fed sucrose compared with those maintained on the control diet. Sucrose intake averaged 6.2 g per rat per d during the experiment.

injection of ^3H -NA at t_0 . At 2, 6, 12 and 24 h after injection, 5–7 rats from each group were killed and their hearts analysed for ^3H -NA and endogenous NA. As shown in the semi-logarithmic plot of cardiac NA specific activity against time (Fig. 1), sucrose feeding was associated with a highly significant ($P < 0.001$) decrease in the calculated half-time of disappearance (t_1), from 14.5 ± 0.5 h in the control group to 8.2 ± 0.1 h in the sucrose-fed group. (Similar differences were observed when cardiac ^3H -NA content was plotted against time.) The fractional NA turnover rate was increased significantly ($P < 0.001$) during sucrose feeding (from $4.80 \pm 0.16\%$ per h in the control group to $8.49 \pm 0.13\%$ per h in the sucrose-fed group). Endogenous levels of cardiac NA decreased significantly ($P < 0.02$) in the sucrose-fed rats compared with the control animals (360 ± 21.7 and 446 ± 25.4 ng NA per heart, respectively). NA turnover rate, calculated as the product of the fractional turnover rate and the endogenous level, was increased in the hearts of sucrose-fed animals (21.4 ± 1.9 and 30.6 ± 2.3 ng NA per heart per h for the 95% confidence intervals in control and sucrose-fed animals, respectively). Sucrose feeding for 3 d, therefore, is associated with increased ^3H -NA turnover.

The effect of inhibition of NA biosynthesis on endogenous levels of NA in hearts of control (normal diet), fasted and sucrose-fed animals is shown in Fig. 2. With both control and sucrose-fed groups significantly ($P < 0.005$ and $P < 0.001$, respectively) decreases in the NA content of rat heart followed 7 h of inhibition of NA biosynthesis; cardiac NA levels were significantly ($P < 0.02$) less in the α MPT-treated control animals, and were similar to the NA levels observed in control animals exposed to cold (4°C). Fasted animals exhibited no change in cardiac NA content after α MPT treatment as previously reported. Thus, two independent methods for assessing NA turnover demonstrate increased NA turnover during sucrose feeding.

Ganglionic blockade interrupts central sympathetic

outflow and as a result decreases peripheral sympathetic activity. The effect of such blockade on endogenous NA and on the residual ^3H -NA remaining in the hearts of control and sucrose-fed animals 10 h after administration of label is shown in Fig. 3. In this experiment chlorisondamine (5 mg per kg, body weight intraperitoneally), a long-acting ganglionic blocking agent, was administered at 5 min, and again at 5 h, after injection of tracer NA into control and sucrose-fed animals. The animals were killed 10 h after the start of the experiment. In the control animals the residual ^3H -NA was 41% ($P < 0.025$) and the endogenous NA 14% (not significant) greater in the group treated with ganglionic blockade compared with the group given saline. In the sucrose-fed animals ganglionic blockade increased residual ^3H -NA 109% ($P < 0.001$) and endogenous NA 23% (not significant). The hearts of sucrose-fed animals without ganglionic blockade contained less ^3H -NA and endogenous NA than did those of the control animals ($P < 0.02$ and $P < 0.05$, respectively) consistent with increased NA turnover while no differences in either residual ^3H -NA or endogenous NA were observed in the hearts of sucrose-fed and control animals with ganglionic blockade. This abolition of differences in cardiac ^3H -NA content between control and experimental groups with ganglionic blockade indicates that increased central sympathetic outflow underlies the accelerated NA turnover observed in the hearts of sucrose-fed animals.

This report, in addition to our previous finding that sympathetic activity is suppressed during fasting, suggests that central sympathetic outflow is influenced by the nutritional state of the animal. The nutritional signal to the central nervous system that leads to the observed alterations in sympathetic activity is unknown. Nutritional modification of central sympathetic outflow may have several important implications. As catecholamines are considered to be the principal mediators of cold-induced thermogenesis^{7,8} and as the mechanisms of thermogenesis are presumed to be similar during cold exposure and with

overfeeding⁹, dietary-induced changes in sympathetic activity may have a central role in nutritionally-determined thermogenesis¹⁰. The physiological importance for cardiovascular disease of the increased sympathetic activity in heart associated with over-feeding is unknown, but dietary alterations in sympathetic activity may be a possible link between the increase in calorie and/or sucrose consumption and the rise in cardiovascular disease observed in Western countries¹¹.

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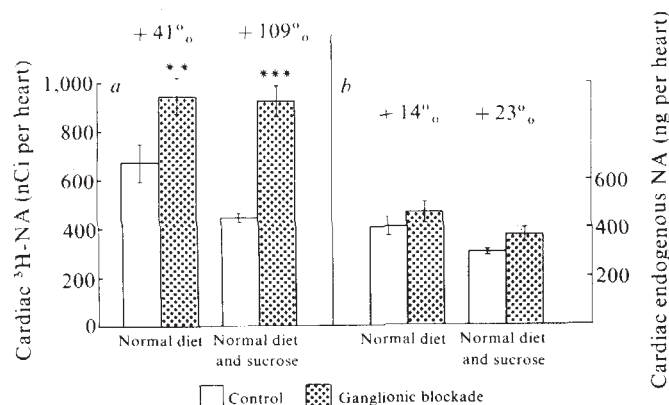
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Occurrence of a new class of tetrahydroisoquinoline alkaloids in L-dopa-treated parkinsonian patients

THE chemotherapeutic administration of massive doses of L-3,4-dihydroxyphenylalanine (L-dopa) with or without peripheral decarboxylase inhibitors to parkinsonian patients can lead to aberrant metabolism which may influence the efficacy of the regime. It is generally agreed that the transformations can include spontaneous chemical reactions facilitated by the inherent reactivity of the catechol nucleus. For example, Sandler *et al.*¹ reported the excretion of tetrahydroisoquinoline alkaloids (TIQs), tetrahydropapaveroline (norlaudanoline) and salsolinol by parkinsonian patients who had consumed large doses of L-dopa and ethanol. The same two TIQs have been detected in brain and urine of mammals administered alcohol and a second compound—for example, L-dopa or pyrogallol; and it has been proposed that these Pictet-Spengler condensation products (of dopamine and dopaldehyde or acetaldehyde, respectively) may be responsible for addiction in alcoholism²⁻⁵. We report here the occurrence of a new class of TIQs, norlaudanolinecarboxylic acids (NLCAs), in the urine of parkinsonian patients on L-dopa with or without carbidopa. Comparative studies with rats on a similar regimen reveal the presence of NLCAs in brain as well as in urine.

NLCAs arise by Pictet-Spengler condensation of phenylpyruvic acids and dopamine (Fig. 1). The parent compound, NLCA, derived from the transamination and decarboxylation products of L-dopa, has been previously characterised and implicated in *Papaver* alkaloid biosynthesis in higher plants^{6,7}. Norcoclaurinecarboxylic acid (NCCA), 3'-O-methylnorlaudanolinecarboxylic acid (MNLCA), and 3',4'-desoxynorlaudanolinecarboxylic acid were synthesised from dopamine and *p*-hydroxyphenylpyruvate, 3-methoxy-4-hydroxyphenylpyruvate and phenylpyruvate respectively, as described for NLCA (ref. 6). Their ultraviolet, nuclear magnetic resonance, infrared and mass spectra were consistent with assigned structures. NLCAs were detected in urine by mass fragmentography on an LKB-9000 gas-liquid chromatograph mass spectrometer. The presence of a carboxyl group dictated a two-step derivatisation of the alkaloids, entailing esterification with

Fig. 3 Effect of ganglionic blockade on *a*, cardiac ^3H -NA and *b*, cardiac endogenous NA in control (normal diet) and sucrose-fed rats. Animals were prepared for this experiment by 3 d of sucrose feeding as in Fig. 1: 5 min and 5 h after the i.v. injection of the ^3H -NA (200 μCi per kg) chlorisondamine (5 mg per kg; Ecolid, Ciba), a ganglionic blocking agent, was administered intraperitoneally. Control animals received similar i.p. injections of diluent. Animals were killed 10 h after injection of the ^3H -NA. Open bars represent saline-treated animals; shaded bars the chlorisondamine groups. Each group contained eight or nine rats. The percentage shown over the bar is the increase over the group without ganglionic blockade. ** Represents $P < 0.025$ and *** $P < 0.001$ compared with control without ganglionic blockade. ^3H -NA and endogenous NA content are significantly lower ($P < 0.02$ and $P < 0.05$, respectively) in the hearts of diluent-treated, sucrose-fed animals compared with diluent-treated, control animals, while no significant differences obtain in control and sucrose-fed animals in the presence of ganglionic blockade. Sucrose intake averaged 8.8 g per rat per d.



pentafluoropropanol followed by acylation of the phenolic and amino groups with heptafluorobutyric anhydride by a modification of a procedure for simple catechol carboxylic acids⁸.

In early experiments single ion monitoring was used and the instrument was focused on the debenzylated fragment (m/e 928), which represented the base peak in mass spectra of all NLCA derivatives. Subsequently the mass spectrometer was interfaced with a Logos Spectrotek computer system which allowed repetitive scanning of the gas-liquid chromatography effluent and data storage. Typical fragment ion current chromatograms generated by the computer from runs with standards and a urine sample are shown in Fig. 2.

The alkaloids were detected by monitoring both the base peak at m/e 928 and benzyl fragment ions (m/e 303 for NCCA, m/e 333 for MNLCA, m/e 91 for 3', 4'-desoxy NLCA). The benzyl fragment ion of NLCA had too low an intensity for routine detection.

With these semi-quantitative techniques the urine of normal subjects (controls) and parkinsonian patients on L-dopa, L-dopa-carbidopa (10:1, Sinemet) or no chemotherapy, were analysed (Table 1). Low but reproducible levels of MNLCA were detectable in urine of L-dopa- and Sinemet-treated parkinsonian patients. NLCA and MNLCA values were obtained for controls and non-treated patients but these were too close to minimal levels of detection to be conclusive. This was also the case for NLCA in treated parkinsonian subjects.

Comparative studies have been carried out with 250 g male Sprague-Dawley rats maintained on 2.5 mg L-dopa or 1.1 mg L-dopa-carbidopa (10:1, Sinemet) per ml drinking water (*ad libitum*) for 1 week. The animals consumed 5–30 mg L-dopa per day and excreted NLCA and MNLCA at a rate of 0.05–48 μ g per 24 h). Brain levels of MNLCA for L-dopa-treated rats averaged 202 ng per g tissue, a value confirmed independently by ion pair reversed phase high-pressure liquid chromatography.

After incubations in physiological conditions (0.2 M phosphate buffer, pH 6.0, at 37 °C for 30 min), 3,4-dihydroxyphenylpyruvate (14 mM) and dopamine (18 mM) reacted to produce 2–3% NLCA in the presence of cell-free homogenates of rat brain tissue. The fact that comparable yields were realised in the absence of brain tissue extracts or with boiled homogenates proved that the condensation was not enzyme dependent. Furthermore, enzymes such as monoamine oxidase, which were demonstrated to be active in these homogenates, did not interfere with the Pictet-Spengler condensation.

In vivo synthesis of MNLCA was demonstrated with rats fed Sinemet for 1 week as described above. The animals were injected intraperitoneally with 0.5 mCi 2,3-³H-L-dopa and killed 1.5–2 h later. Carrier MNLCA (10 mg) was added to *t*-butyl alcohol-ethyl acetate (3:2) extracts of brain

Fig. 1 Pictet-Spengler condensation of dopamine and phenylpyruvates.

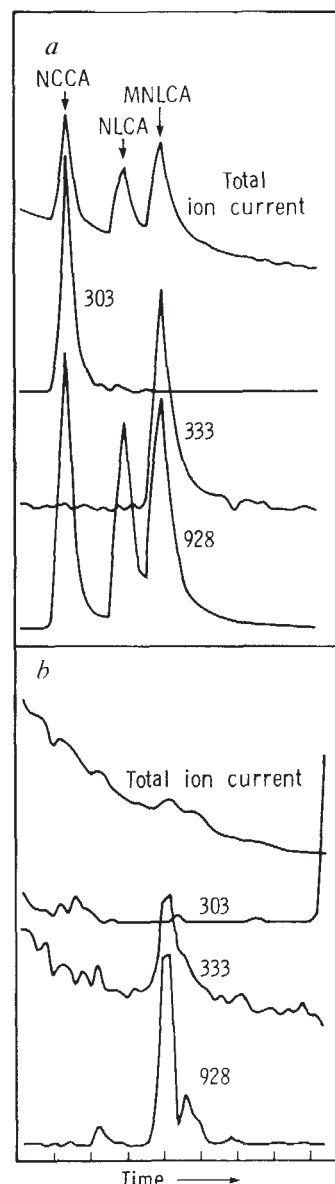
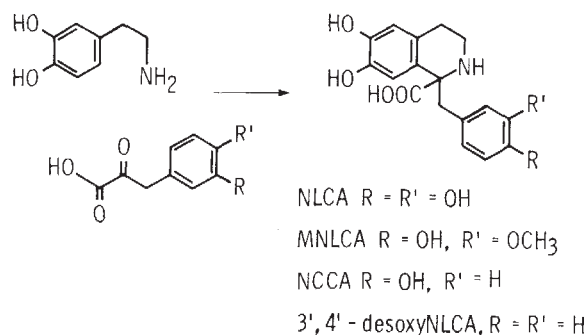


Fig. 2 Fragment ion current chromatograms of derivatised NLCA standards and urine samples by computerised gas-liquid chromatography and mass spectroscopy. Solid NaCl was added to 5 ml urine until saturation, and the solution was extracted with 4 × 5 ml *t*-butyl alcohol-ethyl acetate (3:2). The combined organic phase was evaporated to dryness and taken up in a buffer solution of 0.2 M sodium acetate, pH 8.6, 0.1% EDTA and 1% sodium metabisulphite. Acid-washed alumina (500 mg) was added to this solution and the pH adjusted to 8.6 ± 0.1. This mixture was added to a column of acid-washed alumina (750 mg) pre-equilibrated with buffer and the column was washed with 4 volumes of 1 M HCl. The eluate was saturated with solid NaCl and extracted with 4 × 0.75 ml *t*-butyl alcohol-ethyl acetate (3:2). The combined organic phase was taken to dryness. Recovery of NLCAs in this process ranged from 50–80%. Pre-incubation of urine with glucosylase did not increase yields appreciably. Derivatisation was accomplished by adding to the dry sample 100 μ l 1-H,1-H-pentafluoropropanol-1 (PCR) and 200 μ l heptafluorobutyric anhydride (Aldrich) and heating at 90 °C for 1–3 h. The sample was then taken to dryness with a stream of dry nitrogen gas; 100 μ l heptafluorobutyric anhydride was added and the sample was heated at 90 °C for 15 min. After removal of excess reagent with nitrogen, the sample was taken up in 200 μ l of either anhydrous ethyl acetate or acetonitrile containing 10% (v/v) heptafluorobutyric anhydride. The sample (1–5 μ l) was injected on to a 6-foot silanised glass column (4 mm internal diameter) packed with 3% OV-17 on Gas-Chrom Q (100–120 mesh) in the following conditions: carrier gas, nitrogen; flow rate 30 ml min⁻¹; ion source temperature 290 °C; flash heater 230 °C; column temperature 160 °C; accelerating voltage 2.5 kV; ionising energy 30 or 70 eV; trap current 60 or 120 μ A. Upscans were 4 s and rescans took 1.8 s. The urine sample shown here was obtained from a parkinsonian patient on 1.5 g L-dopa per day. a, Standards; b, urine analysis.

Table 1 Urinary levels of NLCAs in controls and parkinsonian patients

Drug regimen	NLCA (μg per 24 h)	MNLCA (μg per 24 h)	Dopamine (mg per 24 h)
Controls (3)	0	8.4 [0-39]	1.19 $\pm$ 0.27
Sinemet (6)	1.1 [0-7.1]	40.0 [10.9-149.5]	15.2 $\pm$ 3.3
L-Dopa (3)	2.2 [0.6-3.8]	127.7 [55.5-235.9]	204 $\pm$ 66
None (2)	0	3.3 [1.3-5.3]	0.66 $\pm$ 0.18

Urine samples (24 h) were collected from normal subjects (controls) and parkinsonian patients on Sinemet (0.3-0.8 g L-dopa and 30-80 mg carbidopa per day), L-dopa (1.5-4.0 g per day), or no chemotherapy. Urine was stored immediately at -20°C after addition of either 5 ml 6 M HCl or 10 g sodium metabisulphite. TIQ levels did not change with time in these conditions. The values in parentheses indicate the number of subjects used. TIQ values were obtained by mass fragmentography and for MNLCA represent the average of at least two determinations for each individual, with the range of values given in brackets. Dopamine values (mean $\pm$ s.e.m.) were obtained by paired ion high pressure liquid chromatography on a reversed phase column (unpublished results). Levels of 3,4-dihydroxyphenylpyruvate and 3-methoxy-4-hydroxyphenylpyruvate were below the limits of detection of the latter method ($< 0.1 \mu\text{g}$ per ml urine).

and intestinal tissues; it was then recrystallised to constant specific activity⁶. Final specific activities of MNLCA from gut of four different rats were 536-3,342 d.p.m. per mg ($6.5 \times 10^{-4}\%$ incorporation), whereas MNLCA from brain tissue of two rats gave constant specific activities of 231 and 599 d.p.m. per mg ($2.1 \times 10^{-4}\%$ incorporation). Labelled MNLCA was not found in rat liver or kidney in these experiments.

To determine whether endogenous levels of L-dopa metabolites had diluted the tracer, thereby depressing incorporation rates, an experiment was carried out in which an untreated rat was injected intraperitoneally first with carbidopa (1 mg per kg) and 0.5 h later, with 0.5 mCi 2,3-³H-L-dopa. The percentage incorporation ($1.64 \times 10^{-4}\%$) of L-dopa into MNLCA in the gut of this rat did not differ appreciably from the *in vivo* tracer data obtained with Sinemet-treated rats. Rates of *in vivo* conversion of L-dopa to MNLCA were more consistent with a spontaneous chemical reaction than with an enzyme-catalysed reaction. MNLCA formation in gut, in contrast to liver and kidney, reflects the intensive metabolism of L-dopa in the intestine of the rat⁹. Despite the low rates of incorporation, brain levels of MNLCA suggest accumulation can occur, possibly analogous to non-enzymatic melanin formation and deposition in brain.

To determine whether NLCAs can interfere with normal catechol metabolism, they were included in enzyme incubations. Neither 0.1 mM NLCA nor MNLCA had an effect on V_{max} or the apparent K_m of tyrosine aminotransferase (29 nmol per min per mg protein and 2.1 mM, respectively) or L-aromatic amino acid decarboxylase (43 nmol per min per mg protein and 0.60 mM, respectively) in crude rat liver cytosolic fractions. Low concentrations of NLCAs also did not seem to affect hepatic catecholamine catabolic enzymes *in vivo*. If 0.2 mg NLCA, MNLCA or 3',4'-desoxyNLCA and 7-³H-L-noradrenaline (2 μCi , 0.2 nmol) were injected simultaneously into the tail vein of male Swiss albino mice (17-20 g) the percentage ³H label in hepatic normetanephrine and vanillyl glycol was not appreciably different from control experiments in which noradrenaline was injected alone. Specific radioactivities were determined after fractionation by high-pressure liquid chromatography.

The question has been raised as to whether the efficacy of L-dopa chemotherapy in parkinsonism can be attributable to dopamine replacement alone. TIQs have been proposed as possible therapeutic agents¹⁰, whereas some investigators^{11,12} have postulated that they may cause side-effects such as the 'on-off' phenomena observed

clinically in L-dopa chemotherapy. These hypotheses imply interaction of TIQs with catecholaminergic receptors, and evidence exists for transmitter-like behaviour of simple aldehyde-derived TIQs⁴. Thus, the occurrence of NLCAs in human urine and rat urine and brain demonstrated here, provides a basis for further investigation of theories concerning the role of TIQs in parkinsonian chemotherapy.

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γ Endorphin, α endorphin and Met-enkephalin are formed extracellularly from lipotropin C fragment

A SERIES of peptides derived from lipotropin has been reported to possess morphinomimetic properties. The C fragment (or β endorphin, residues 61-91) was isolated from pituitary^{1,2} and shown to be present in brain³, the C' fragment (61-87) occurs in pituitary¹, γ endorphin (61-77) and α endorphin (61-76) were obtained after acid extraction of tissue of hypothalamic and pituitary origin⁴, and Met-enkephalin (61-65) was found in extracts of brain^{5,6}. Examination of the ability of these peptides to displace specifically bound ³H-naloxone from brain opiate receptors showed that C fragment was much more potent than the shorter peptides⁷ and investigation of the antinociceptive properties showed that C fragment alone produces profound and long-lasting analgesia⁸⁻¹¹. In addition, various other central activities are exhibited by C fragment¹²⁻¹⁴. We report here that C fragment can be degraded by membrane bound enzymes from rat brain to form the smaller opiate-like peptides.

The use of iodine-labelled peptide, with the radiolabel on the NH₂-terminal tyrosine, ensured that the substrates could be studied at near physiological concentration and products containing the NH₂-terminal region of C fragment were readily identified. Membranes from rat brain were prepared by extensive washing of the synaptosomal fraction (P₂) described by Whittaker *et al.*¹⁵. Incubations of [¹²⁵I]C-fragment (100,000 d.p.m.; 2 Ci mmol⁻¹) with membrane preparations (0.42 mg per ml) were performed in 10^{-3} M bacitracin for 16 h at 37 $^\circ\text{C}$ in 1 ml of 0.05 M sodium chloride-0.1 M sodium phosphate at pH 7.4 or 0.1 M ammonium acetate at pH 5 and the suspensions were gently agitated. The products were resolved by gel filtration on Sephadex G-50 in 50% (v/v) acetic acid and chromatography on thin layers of cellulose MN400 with *n*-butanol-pyridine-acetic acid-water (15 : 10 : 3 : 12; v/v) or *n*-propanol-0.2 M ammonium hydroxide (3 : 1; v/v). Shorter peptides (LPH 61-67 or 61-68, 0.34 mM) were incubated with membranes in similar conditions for 1 h and the mixtures were fractionated by

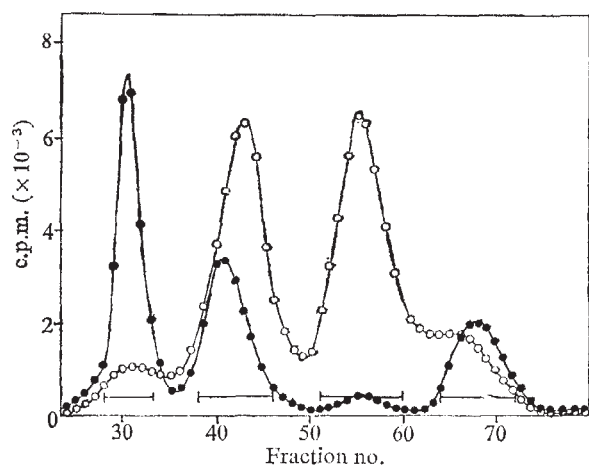


Fig. 1 Gel filtration of the products of digestion of [125 I] C fragment with membrane-bound proteases from brain. Brain digests were prepared as described in the text. After addition of an equal volume of acetic acid, membranes were removed by centrifugation. The supernatant was concentrated *in vacuo* at 40 °C and gel filtration carried out on a 150 $\times$ 1 cm Sephadex G-50 column in 50% (v/v) acetic acid; fractions (1.8 ml), flow rate of 6 ml h $^{-1}$. ●, Elution profile obtained after digestion at pH 7.4; ○, profile obtained at pH 5.0. Fractions pooled as indicated by the horizontal bars were evaporated to small volume and portions were subjected to thin layer chromatography together with reference peptides¹⁶. Fractions 28–33 contained residual C fragment, fractions 38–46 contained only γ endorphin (pH 7.4) or α endorphin together with 61–73 (pH 5), fractions 52–60 contained Met-enkephalin (pH 7.4) and 61–68 (pH 5) and fractions 64–73 contained tyrosine.

gel filtration on Sephadex G-25 in 50% acetic acid and preparative electrophoresis on paper. Identification of the reaction products was by amino acid analysis.

At pH 7.4 in the presence of bacitracin (Fig. 1) the main product formed from C fragment was γ endorphin (61–77); there was in addition a small amount of Met-enkephalin (61–65). At lower pH values, C fragment was converted rapidly and in high yield to α endorphin (61–76) and Met-enkephalin. The heptapeptide (61–67) generated Met-enkephalin and the dipeptide Thr-Ser readily even at neutral pH.

The endopeptidase activity, assayed by release of Met-enkephalin from the heptapeptide 61–67, was associated with membranes but the enzyme could be detected in intact as well as lysed synaptosomal preparations. Moreover, the enzyme did not co-purify with the membrane components carrying the opiate receptors. When extensively washed synaptosomes were lysed and the membrane fragments resolved on sucrose density gradients, the membranes with affinity for 3 H-dihydromorphine were concentrated in the central fractions of the gradient whereas the endopeptidase activity was distributed uniformly. The results indicate that the endopeptidase is not an intrinsic component of the opiate receptor complex.

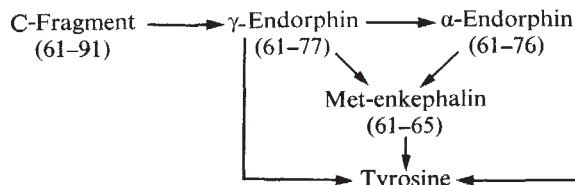
On incubation with striatal slices, [125 I]C-fragment underwent slow degradation with a half life of 3.2 h whereas the half life of [125 I] γ endorphin was 1.1 h. Residual peptide was determined by chromatography of the medium on Sephadex G-50 (Fig. 2). With C-fragment no stabilisation occurred on addition of bacitracin but the aminopeptidase inhibitor had a pronounced effect on the degradation of γ endorphin. The results show that the degradation of C fragment in striatum is not initiated by the attack of an aminopeptidase. The first step, as with the proteolysis catalysed by brain membranes, must involve attack by an endopeptidase.

The specificity of the endopeptidase was determined by examination of the soluble fraction of the incubation mixture. Gel filtration on Sephadex G-50 showed that the disappearance of C fragment was accompanied by the appearance of peptide fractions of lower molecular weight which retained the NH $_2$ -

terminal region of C fragment (Peaks I and II, Fig. 2). The formation of fraction I was markedly enhanced in the presence of bacitracin and ion-exchange chromatography showed that the major product was γ endorphin (Fig. 3). While the yield of γ endorphin was increased by bacitracin, continued incubation with the aminopeptidase inhibitor led to the formation of α endorphin in addition to γ endorphin. Thus, although C fragment is resistant to the action of aminopeptidases¹⁶ and carboxypeptidases¹⁷, γ endorphin is more susceptible to both enzymes.

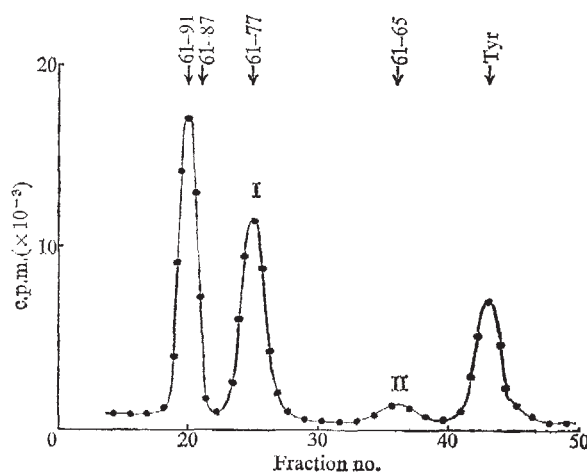
A small percentage of short peptides retaining the NH $_2$ -terminal section of C fragment was observed during the gel filtration on Sephadex G-50 (Peak II, Fig. 2). Resolution of this fraction showed that the major component was Met-enkephalin and there was also some hexa- or heptapeptide (Fig. 4). Even though the small peptides are susceptible to degradation by exopeptidases, their formation was not greatly enhanced by the addition of bacitracin. It seems that the endopeptidase cleavage of γ and α endorphin proceeds only with difficulty.

The degradation of C fragment by membrane-bound enzymes and by striatal slices takes place in discrete stages, forming γ endorphin, α endorphin and Met-enkephalin. The accumulation of these peptides is strongly favoured by the presence of bacitracin or by acid pH, conditions which prevent the loss of the NH $_2$ -terminal tyrosine.



The degradation seems to be mediated by extracellular enzymes and does not involve a tissue uptake system because

Fig. 2 Gel filtration of [125 I] C fragment incubated with striatal slices. Coronal slices (about 200 mg wet weight) through the corpus striatum were prepared by section of fresh rat brains 3.5 mm and 2.5 mm rostral to the anterior part of the optic chiasma. The slices were pre-incubated (45 min, 37 °C) in 3 ml of saline (NaCl, 134 mM; KCl, 5 mM; KH $_2$ PO $_4$, 1.25 mM; MgSO $_4$, 2.0 mM; CaCl $_2$, 1 mM; NaHCO $_3$, 16 mM; glucose, 10 mM) containing 10 $^{-4}$ M bacitracin. The slices were transferred and gently agitated at 37 °C in 3 ml of saline, or 3 ml of saline and 10 $^{-4}$ M bacitracin, containing 6 $\times$ 10 6 c.p.m. of iodinated C fragment (about 2 Ci mmol $^{-1}$). After 4 h incubation an equal volume of glacial acetic acid was added to the supernatant and gel filtration was performed on Sephadex G-50 (150 $\times$ 0.9 cm) in 50% (v/v) acetic acid. The eluate fractions (2.2 ml) were monitored for radioactivity. The arrows indicate the elution positions of authentic 125 I or 125 I-labelled peptides.



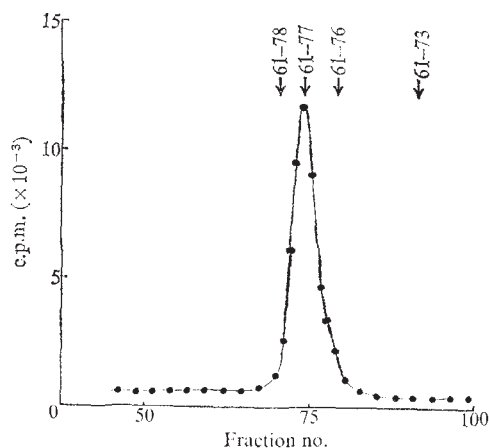


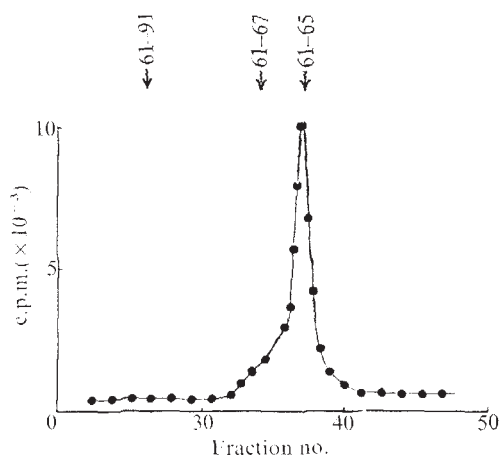
Fig. 3 Identification of γ endorphin as the first degradation product formed from C fragment in striatal slices. The peptide fraction Peak I, obtained by gel filtration of [125 I] C fragment incubated with a striatal slice in the presence of bacitracin, was concentrated *in vacuo* and chromatographed together with [131 I] γ -endorphin on SP Sephadex C-25 in 0.05 M phosphate pH 4.5 with a linear gradient from 0 to 0.5 M NaCl (200-ml mixer). The elution positions of the 61-73, 61-76, 61-77 and 61-78 peptides were established by chromatography of standard mixtures containing [125 I]- or [131 I]-labelled peptides.

no enrichment of labelled peptide could be detected in the slices; but the results do not preclude the possibility that formation of Met-enkephalin and the endorphins might also occur intracellularly.

Our results show that the extracellular metabolism of C fragment takes place in a specific manner to form the series of opiate-like peptides that have been isolated from brain. This may mean that the shorter peptides that have been isolated are degradation products of C fragment rather than natural peptides. It is, however, too early to be certain about this since it has been reported that enkephalin is present in rat brain even after rapid killing by microwave irradiation¹⁸.

We thank Dr A. F. Bradbury for synthesising Met-enkephalin

Fig. 4 Identification of Met-enkephalin as the principal component of the small peptide fraction formed by degradation of C fragment in striatal slices. The peptide fraction Peak II, obtained by gel filtration of [125 I] C fragment incubated with a striatal slice in the presence of bacitracin, was evaporated *in vacuo* and chromatographed on Sephadex G-15 (150 $\times$ 0.9 cm) in 50% (v/v) acetic acid, fraction size 1.4 ml. The column was calibrated with [125 I] C fragment, the heptapeptide (61-67) and Met-enkephalin (61-65).



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Identification of the herpes simplex virus DNA polymerase gene

THERE is at present much interest in the herpes viruses because they have been associated with human cancer and cause latent infections with recurrent disease. They are also of interest because they form a relatively simple but apt model for the processes of transcription and DNA replication in eukaryotic cells. We have studied the virus-induced proteins involved in DNA replication, and present here results that give definitive evidence that the virus-induced DNA polymerase is encoded in the virus genome, that locate the gene for virus DNA polymerase, and also show that the enzyme is essential for virus replication.

Keir and Gold¹ demonstrated that herpes simplex virus (HSV) induced a DNA polymerase and Keir *et al.*^{2,3} showed that the enzyme was stimulated by high salt concentrations and inhibited by antiserum to virus-infected cells. Weissbach *et al.*⁴ demonstrated partial purification of the enzyme and more recently, we have purified the enzyme to the point where a virus-induced protein of molecular weight 149,000 can be associated with this activity⁵. Aron *et al.*⁶ and Purifoy and Benyesh-Melnick⁷ showed that HSV mutants in several complementation groups were deficient in induction of the DNA polymerase and, further, that some of these mutants induced an enzyme which was temperature sensitive *in vivo* but not *in vitro* in crude extracts.

We have been studying the DNA polymerase induced by one of these temperature-sensitive mutants of HSV type 1 (HSV-1) which is designated tsD9 (ref. 8) and by a spontaneous ts⁺ revertant of this mutant, designated DR3 (ref. 9). The purification of the enzyme from HEp-2 cells infected with this mutant at the permissive temperature is shown in Fig. 1. The details of this purification scheme and its evaluation will be published elsewhere, but suffice to say here that the mutant enzyme behaves exactly as the wild-type enzyme during purification, both in chromatographic behaviour and in the amounts of the starting

enzyme recovered (10–15% overall yield). Enzyme was purified in precisely the same manner both from cells infected with the wild-type virus (HSV-1 strain KOS) and from cells infected with the revertant, DR3. All the enzymes were neutralised specifically by antiserum to virus-infected cells.

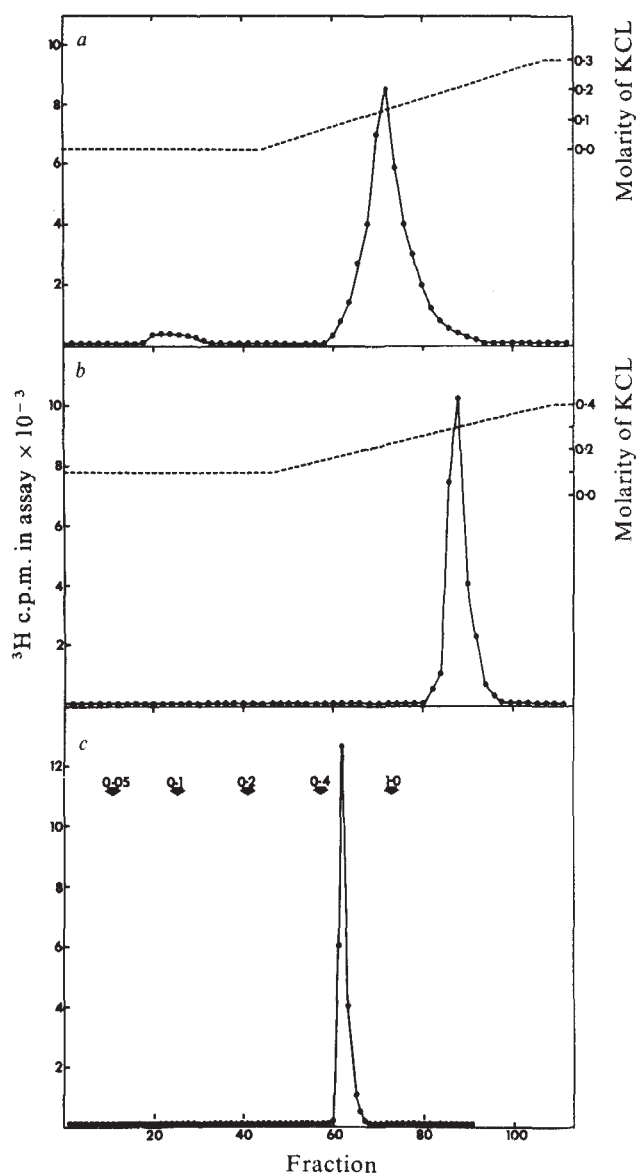


Fig. 1 Purification of virus-induced DNA polymerase from tsD9-infected cells. Enzyme was extracted from virus-infected cells as described previously¹³, using a high salt procedure. Total enzyme activity was extracted, dialysed against 50 mM Tris-HCl, pH 7.5, 20% glycerol, 0.2% NP40 and 0.5 mM dithiothreitol (DE buffer), and applied to a DEAE-cellulose column equilibrated in the same buffer. The enzyme was adsorbed to the column and then eluted with DE buffer containing 0–0.3 M KCl. DNA polymerase activity was detected in column fractions using our standard assays for the virus-induced enzyme containing 100 mM KCl in the reaction mixture⁷. The enzyme eluted at between 0.1 and 0.15 M KCl as shown (a). The partially purified enzyme was diluted in DE buffer and applied to a phosphocellulose column equilibrated in the same buffer and prewashed with 500 $\mu\text{g ml}^{-1}$ bovine serum albumin (BSA). The column was developed with DE buffer containing 0.1–0.4 M KCl and the enzyme eluted at 0.25–0.3 M KCl (b). The activity peak from this column was dialysed against DNA cellulose buffer (DNB, 50 mM Tris-HCl, pH 7.5, 50 mM KCl, 20% glycerol, 0.2% NP40, 0.5 mM dithiothreitol, 1 mM EDTA) after adjusting the protein concentration to 500 $\mu\text{g ml}^{-1}$ with BSA. The dialysed enzyme was applied to a DNA-cellulose column equilibrated with DNB and eluted in stepwise fashion with 0.05, 0.1, 0.2, 0.4, and 1.0 M KCl in the same buffer. Purified enzyme eluted with 0.4 M KCl (c) and was then used for further experiments.

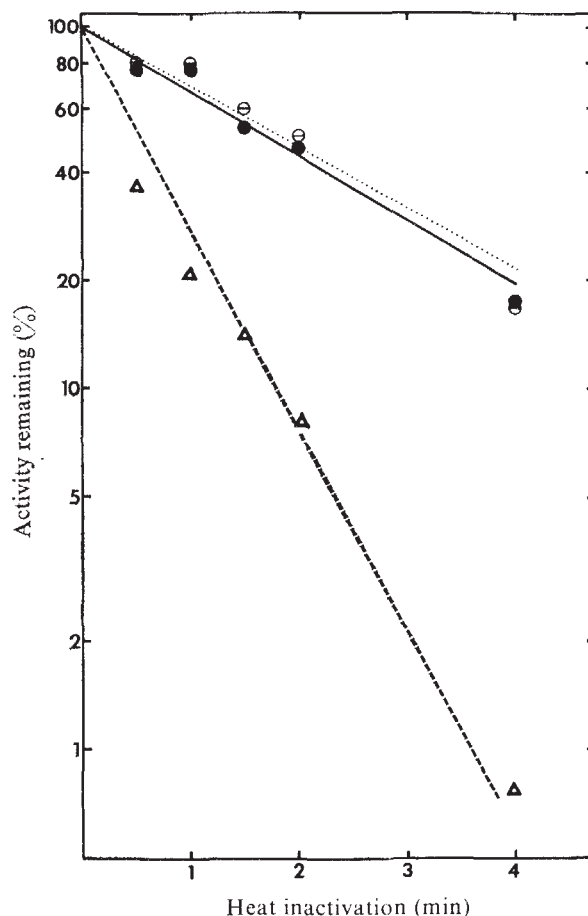


Fig. 2 Heat inactivation of purified enzymes. DNA polymerase purified from wild-type (●), mutant tsD9 (Δ), or revertant DR3 (○) infected cells was heated at 39 °C for various times in reaction mixture, then template DNA was added and the residual DNA polymerase activity determined using standard assay methods. The specific activity of the enzymes was between 4,000 and 5,000 units mg^{-1} (1 unit is defined as 1 nmol TTP incorporated h^{-1}). One hundred per cent activity corresponds to 70,000 c.p.m. in this assay for each enzyme.

The next step was to test the thermal inactivation of the purified enzymes from mutant, revertant, and wild-type virus-infected cells in the absence of DNA template. In these experiments, purified enzyme (specific activity approximately 4,500 units mg^{-1}) was incubated at 39 °C in reaction mixture without DNA for various periods, template was added and the residual DNA polymerase activity determined. The results presented in Fig. 2 demonstrate that DNA polymerase induced by wild-type virus and revertant is considerably more stable than that induced by the mutant. This result has been verified with batches of enzyme from several independent purifications. Thus, the temperature-sensitive mutation in tsD9 directly affects the DNA polymerase induced by the mutant, causing a loss in the stability of the enzyme; reversion of the mutation is accompanied by recovery of that stability.

HSV replication has been shown to be inhibited by the drug, phosphonoacetic acid¹⁰. Further mutants of the virus have been isolated which are resistant to the drug, and this resistance correlates with DNA polymerase activity in crude extracts *in vitro* (refs 11 and 12). TsD9 was not selected for phosphonoacetic acid resistance and yet is resistant to the drug (ref. 9). To confirm this observation and further to correlate the sensitivity or resistance of the enzyme to phosphonoacetate, it was essential to test

purified enzyme. This experiment has been done and the results are shown in Fig. 3. Purified DNA polymerase from wild-type or DR3 (revertant)-infected cells was extremely sensitive to the drug: 50% inhibition of the enzyme activity was observed at a drug concentration of $0.5\text{--}1\text{ }\mu\text{g ml}^{-1}$. With the purified mutant enzyme a 10–20-fold higher concentration of the inhibitor was required to inhibit the enzyme activity. Reversion of the temperature-sensitive lesion again resulted in restoration of wild-type characteristics to the mutant enzyme.

Our results demonstrate that the temperature-sensitive mutation in the HSV-1 tsD9 genome elicits altered characteristics of the virus-induced DNA polymerase, namely temperature sensitivity and phosphonoacetic acid resistance. Since tsD9 has been located on the virus genetic map between tsO22 and tsN20 (P. A. Schaffer, personal communication), our results enable the virus DNA polymerase gene to be positioned on the current genetic map. Marker rescue with restriction enzyme fragments of tsD9 will enable the location of the DNA coding for the enzyme on the physical map of virus DNA. Several other conclusions can be drawn from the data. First, the virus codes for the induced DNA polymerase, which is the first direct evidence for an animal virus specified DNA-dependent DNA polymerase. We suggest that this polymerase be designated pol H, to distinguish it simply from host cell enzymes. Second, the DNA polymerase activity is essential for virus DNA replication and growth, since tsD9 does not replicate DNA at the non-permissive temperature. Third, phosphonoacetate resistance, at least in this case, is conferred on the virus by changes in the structure of its DNA polymerase—

thus providing a useful method for selection of temperature-sensitive mutants in the viral enzyme.

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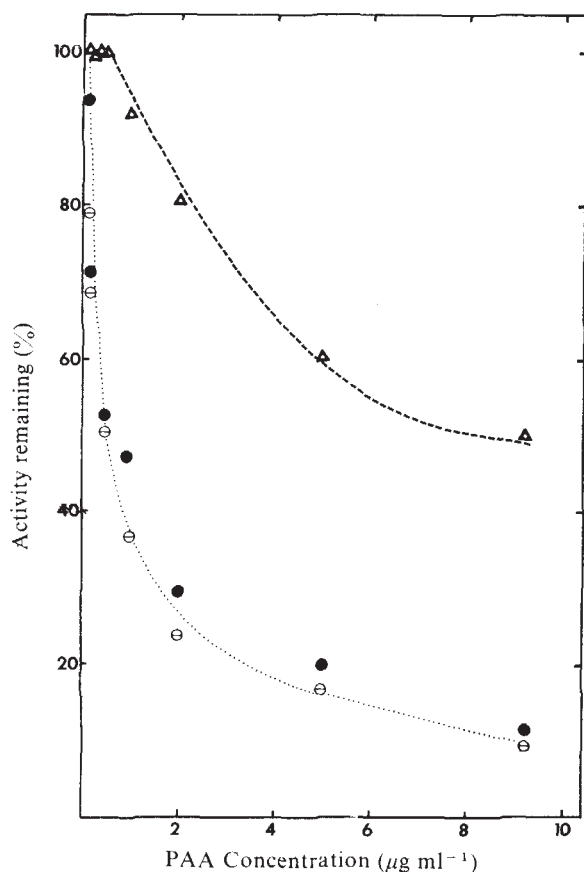
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Fig. 3 Phosphonoacetic acid (PAA) sensitivity of purified enzymes. DNA polymerase purified from wild-type (●), mutant tsD9 (△), or revertant DR3 (○) infected cells was assayed in the presence of various concentrations of PAA. One hundred per cent activity corresponds to about 120,000 c.p.m. in this assay.



Sister chromatid exchange and chromatid interchange as possible manifestation of different DNA repair processes

SISTER chromatid exchange (SCE) is a symmetrical exchange between a newly duplicated chromatid and its sister¹. The demonstration that many agents that damage DNA also increase the frequency of SCE² suggests that SCE is a reflection of a basic DNA repair process, and perhaps a cytological manifestation of post-replication repair of a recombinational character^{2–6}. The question then arises as to the correlation between SCE and chromatid aberration, particularly chromatid interchange (CI), which also occurs as a consequence of exchange between newly duplicated chromatids^{7,8}. To investigate whether these two types of exchange are due to the same molecular event, I have studied the reaction of SCE and CI to caffeine and cycloheximide in endoreduplication mitoses of cultured human embryonic skin fibroblasts. I report here evidence suggesting that the events involved are separate.

Endoreduplication is an internal doubling of chromosomes resulting from two successive DNA synthesis periods without intervening cytokinesis. Sister chromosomes are paired to form a diplochromosome. When the cells are grown for two replication cycles in the presence of 5-bromodeoxyuridine (BUdR) so that chromosomes can be stained differentially to distinguish one chromatid unifarly with BUdR from its sister bifarly substituted, three types of exchange can be seen—twin SCE occurring in the first S phase, single SCE occurring in the second S phase and intradiplochromatid interchange (IDCI) which occurs between chromosomes in a diplochromosome (Fig. 1).

Fibroblast cultures established from skin fragments of 9–11-week-old human abortuses were used at passages 4–7. Confluent cultures were trypsinised and the cells were grown in Leibovitz's L-15 medium containing 15% foetal calf serum and 20 μM BUdR. Cells were fixed for chromosome

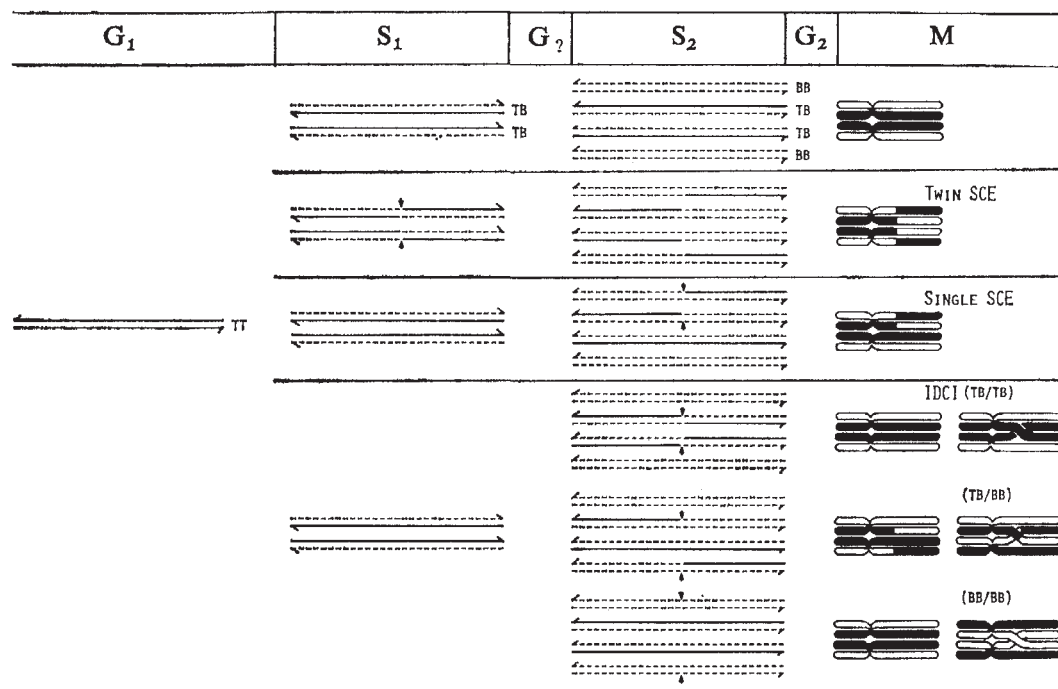
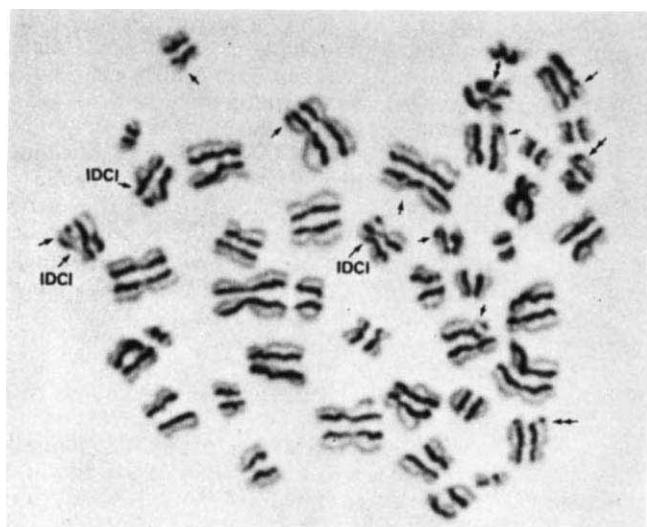


Fig. 1 Diagram of SCEs and IDCIs in diplochromosomes and orderly segregation of DNA strands during the replication cycle. The diagram is based on the unine model of chromosomes, in which a chromatid contains a single DNA duplex. The new DNA strands always segregate outside the parental strands. When the cells are grown in the presence of BUdR for two replication cycles, except for disruption by exchanges, the chromatids of unifilar BUdR-substitution (TB) are on the inner side in diplochromosomes while those of bifilar BUdR-substitution (BB) are on the outer side. Broken lines represent BUdR-substituted DNA strands and solid lines unsubstituted grand-parental strands. Arrows show the sites of exchanges.

preparations 70 h after plating—mitotic arrest was achieved by a 4-h prefixation with colcemid. The effects of caffeine and cycloheximide were studied by treating cells for the last 24 h. In some cultures, 0.005% β -mercaptoethanol was added to increase the frequency of endoreduplication mitoses. Chromosome preparations were processed for differential chromatid staining according to the 'fluorescent plus Giemsa' technique of Perry and Wolff⁹, and endoreduplication mitoses (Fig. 2) were analysed for types and frequencies of SCEs and IDCIs.

Figure 3 shows that incubation in the presence of β -mercaptoethanol increased the frequency of twin and

Fig. 2 Endoreduplication mitosis with differential chromatid staining showing single SCEs (single arrows), twin SCEs (double arrows) and IDCIs. The cell was exposed overnight to a black-light fluorescent lamp (Toshiba FL20BL) in 33258 Hoechst $1 \mu\text{g ml}^{-1}$, incubated in distilled water for 2 h at 62 °C and stained with Giemsa. Bifilarly BUdR-substituted chromatids are stained light while unifilarly substituted ones are stained dark.



single SCEs but did not affect that of IDCIs. In the untreated cultures, either with or without β -mercaptoethanol, the rates of SCE in the first and second S phases were approximately the same, the number of twin SCEs per diplochromosome not being significantly different from the number of single SCEs per chromosome. Because the treatment for the last 24 h covers only the second replication cycle in my culture conditions⁹, twin SCEs related with the first S phase could not be influenced by caffeine and cycloheximide treatment during this interval. But exchange events relating to the second S phase, that is, single SCEs and IDCIs, were significantly affected by these compounds, although in different ways—caffeine reduced IDCIs but increased single SCEs, whereas cycloheximide had no effect on IDCIs but suppressed single SCEs. The involvement of chromatids in the formation of IDCIs was non-random. Of 565 IDCIs scored, 518 (91.7%) were exchanges between chromatids with uni- and bifilar substitution (TB/BB type) (Fig. 1), and the TB/TB and BB/BB types were unexpectedly infrequent, constituting only 7.8% and 0.5%, respectively. The IDCIs of TB/TB and BB/BB types between chromatids with identical staining properties, could only be recognised morphologically as X-shaped criss-cross exchanges. But among IDCIs of TB/BB type, 91.3% had the same configuration of overlapping chromatids. Thus the predominance of TB/BB exchanges cannot be explained by different rates of detection, but suggests some biological significance for the formation of IDCIs in these conditions. In the cells grown in the absence of BUdR, the frequency of morphologically identifiable IDCIs was 0.02 per diplochromosome, about a quarter of that in the presence of 20 μM BUdR, which apparently induces IDCIs.

It is interesting that IDCIs can be suppressed by caffeine. Caffeine inhibits post-replication repair¹¹⁻¹³, which is thought to involve the formation of gaps in the daughter strand during replication on a damaged DNA template and the filling in of these gaps by *de novo* synthesis¹⁰. Similar processes and/or types of lesion are likely to be responsible for CI formation, possibly through a recombinational event. In eukaryotic cells, cycloheximide causes immediate inhibition of DNA replication¹⁷⁻¹⁹. Although the action of cycloheximide may be diverse²⁰⁻²¹, it is tempting to correlate the

cycloheximide-sensitive SCE with some components of the DNA replication process.

Although my study concerned chromatid exchange in diplochromosomes, it is possible that the process responsible for IDCI formation occurs during normal diploid mitosis. This would manifest as false SCE²²⁻²³ when occurring between sister chromatids and would give rise to CI and other chromatid exchange aberrations if chromosome segments involved are closely positioned in the nucleus and replicate at a similar time. In spite of the general association between SCEs and chromatid aberrations in their response to DNA-damaging agents, there is evidence against a correlation between two phenomena. Among human genetic diseases known to be characterised by a high frequency of spontaneous chromatid aberrations, only Bloom's syndrome involves apparent association of the two, showing an abnormally high rate of spontaneous SCE²⁴. But in

Fanconi's anaemia²⁵, ataxia telangiectasia²⁶ and incontinentia pigmenti (my unpublished data) the rate of SCE is normal. Moreover, in Fanconi's anaemia²⁵ and xeroderma pigmentosum²⁷, sensitivity to SCE caused by chemical mutagens does not correlate with the sensitivity to chromatid aberration formation, whereas IDCI formation does²⁸. Also the effects of caffeine on chemical induction of SCE differ in plants²⁹ and Chinese hamster cells¹. These lines of evidence reinforce my conclusion that there is a two-step, replication-mediated, repair mechanism and that SCE and CI are cytological manifestations of them. The relative potential of each process may vary with the types of DNA damage, repair systems and organisms involved.

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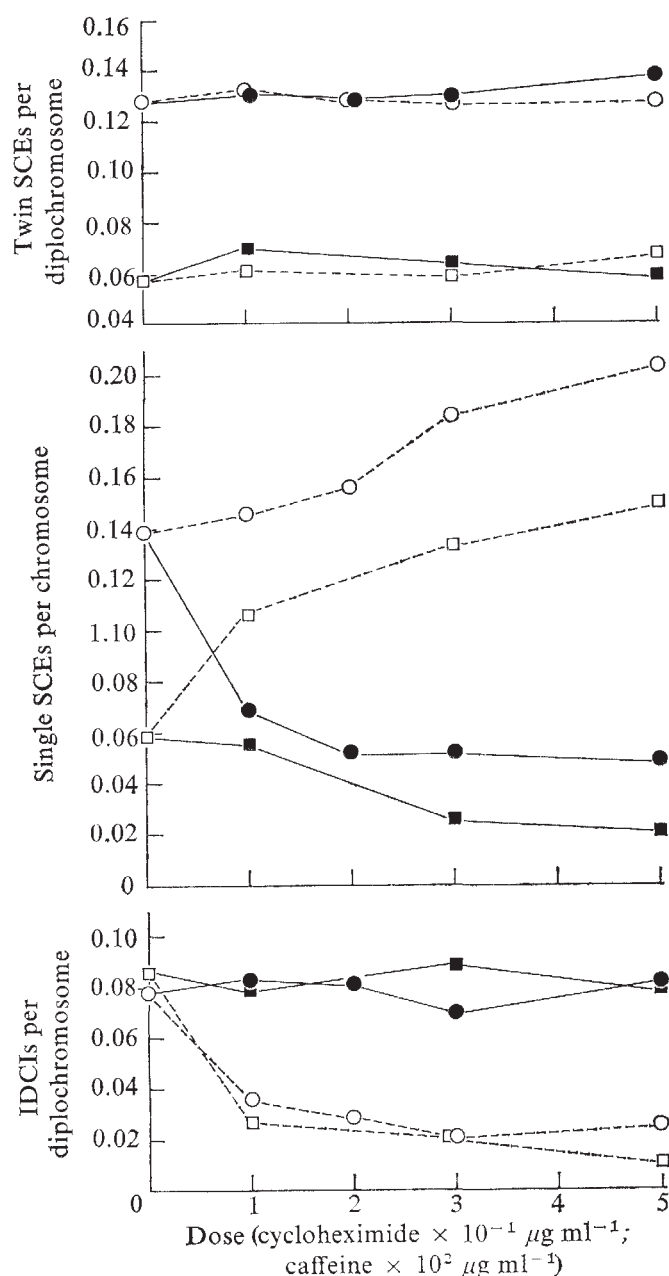
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Long-term persistence of O⁶-methylguanine in rat brain DNA

THE carcinogenic potency of alkylating agents correlates with the extent of their reaction at oxygen atoms in DNA bases¹. O⁶-Alkylguanine is amongst those derivatives most likely to be involved in the initiation of malignant transformation since it leads to mispairing *in vitro* and thus can be considered promutagenic^{2,3}. Repair excision of O⁶-alkylguanine from DNA has been demonstrated in bacteria⁴ and mammalian cells⁵, and seems to be initiated by a specific N-glucosidase⁶. In the intact animal, the persistence of O⁶-alkylguanine in DNA of different tissues varies considerably. In rats, the induction of neural^{7,8} and renal⁹ neoplasms correlates with a deficient excision capacity of the respective target tissues. O⁶-Alkylguanine produced by a single injection of the neuro-oncogenic agents N-ethyl-N-nitrosourea and N-methyl-N-nitrosourea (MNU), is removed

Fig. 3 Effects of caffeine and cycloheximide on frequencies of SCE and IDCI. Experiments were done in the presence (circles) or absence (squares) of β -mercaptoethanol. Solid symbols, cycloheximide, open symbols, caffeine. Each point is based on 12-25 endoreduplicated mitoses.



from brain DNA significantly more slowly than from that of liver and kidney, the half life in cerebral DNA being in the range of 9–11 d for both *O*⁶-ethyl- and *O*⁶-methylguanine^{7,10}. These data are, however, based only on observations for up to 10 d. We report here that the decay curve for *O*⁶-methylguanine in brain DNA consists of different components and that this modified base may persist in cerebral DNA for a considerable fraction of the animal's life.

Adult female BD-IX rats (100–120 g) received a single intravenous injection of *N*-[³H]-methyl-*N*-nitrosourea (25.5 mCi mmol⁻¹) at a dose of 10 mg per kg body weight. After time intervals ranging from 4 h to 184 d, DNA was isolated by phenol extraction¹¹ from the combined organs of two animals. Following hydrolysis in 0.1 M HCl (37 °C, 24 h) purine bases were separated on Sephadex G-10 columns (1 × 90 cm) using 0.05 M ammonium formate (pH 6.2) as eluent. The amounts of methylated purines were expressed as a fraction of the parent base, assuming that the specific radioactivity of the methyl group was identical to that of the injected carcinogen¹².

The persistence of *O*⁶-methylguanine in DNA of various rat organs is shown in Fig. 1. Excision from hepatic DNA was faster than from that of any other tissue, *O*⁶-methylguanine concentrations being below the level of detectability within 7 d. In lung and kidney DNA, the limit of detection was reached by 28 and 84 d respectively. In brain, the rate of excision was considerably slower. Following a rapid decrease during the first hours after injection, there is a distinct slope extending from 12-h to 14 d. During this period, *O*⁶-methylguanine values were reduced from 78% to 50% of the initial (4 h) concentration. Subsequently, the rate of removal decreased greatly. In contrast to the other organs and to the initial part of the decay curve for the nervous system, this latter component was linear. At 184 d, 25% of the initial concentration was still present in cerebral DNA. Extrapolation of these data suggests that complete removal of *O*⁶-methylguanine would require approximately 1 yr.

We can rule out the possibility that tissue-dependent variations in cell turnover significantly account for the observed differences in the rate of loss of *O*⁶-methylguanine from DNA. 7-Methylguanine which is removed non-enzymatically from DNA by hydrolysis, shows a similar decay in both liver and brain (Fig. 2).

The dose of MNU used in this study (10 mg per kg) represents approximately 10% of the 50% lethal dose (LD₅₀) and is not sufficient to induce tumours. Given

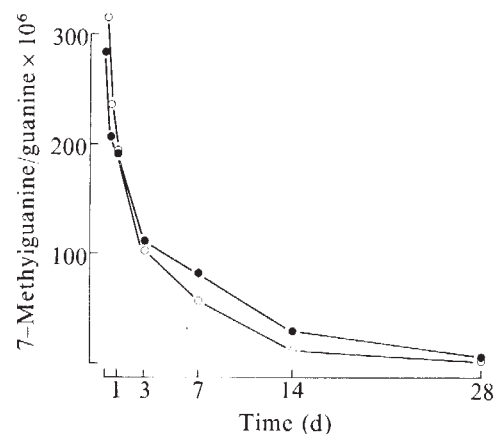


Fig. 2 7-Methylguanine concentrations in DNA of rat brain (●) and liver (○) following a single injection of ³H-MNU. Experimental details as in Fig. 1.

repeatedly, however, it will produce primarily tumours of the nervous system. In contrast to other organs, such multiple doses lead to a linear increase of *O*⁶-methylguanine in brain DNA¹¹. It now seems that this additive effect is based on the linear (slow) segment of the excision curve. It is possible, however, that different excision kinetics apply to alkylated bases resulting from environmental exposure to very small doses. In rat liver, the rate of excision has indeed been shown to be inversely related to the initial amount of *O*⁶-methylguanine produced^{13,14}.

The different components of the *O*⁶-methylguanine removal curve in rat brain may represent DNA fractions within the chromatin complex having a different accessibility to repair enzymes¹⁵. Alternatively, the different slopes may reflect the repair capacity of different brain cell populations, for example neuronal versus glial cells. The latter possibility is being investigated. If the persistence of *O*⁶-alkylguanine in DNA leads to mutational events responsible for malignant transformation, the slow component should represent glial DNA, since the tumours induced by MNU and related carcinogens are almost exclusively of glial origin.

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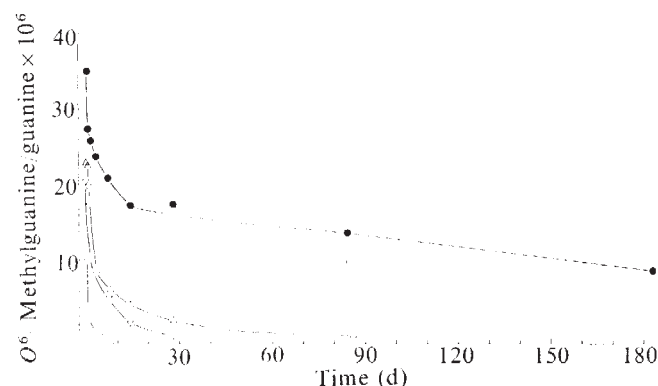
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Fig. 1 Persistence of *O*⁶-methylguanine in DNA of brain (●), kidney (△), lung (▽), and liver (○). Female BD-IX rats received a single intravenous injection of *N*-[³H]-methyl-*N*-nitrosourea (10 mg kg⁻¹) and were killed at time intervals ranging from 4 h to 184 d.



Daunorubicin and adriamycin facilitate actinomycin D binding to poly(dA-dT) poly(dA-dT)

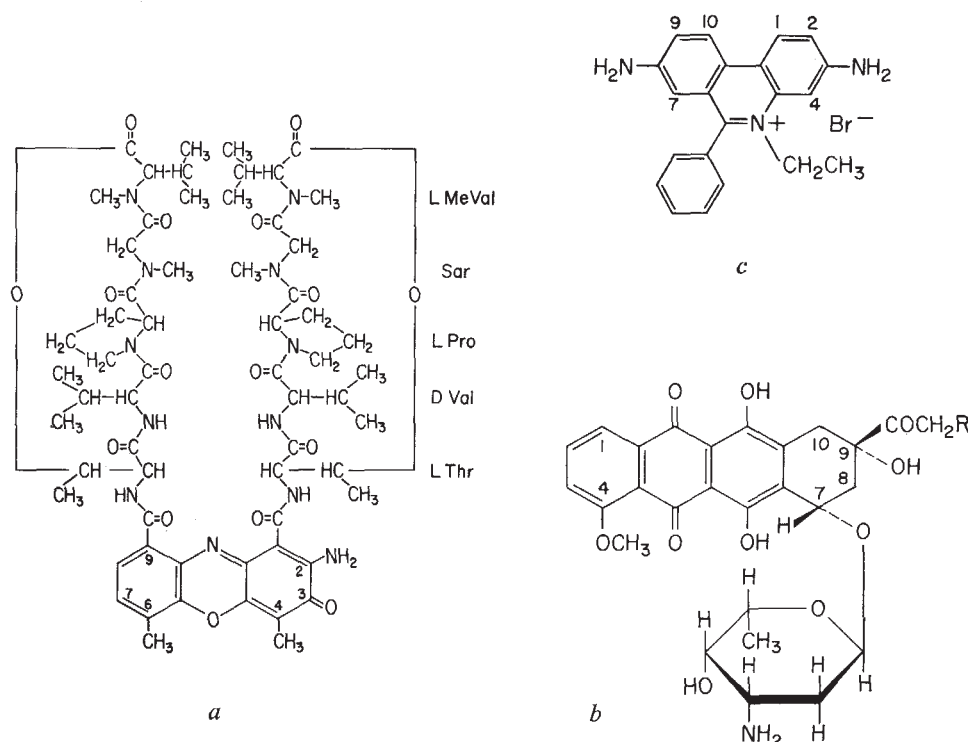
ACTINOMYCIN D (Fig. 1a) binds to DNA by intercalation of the phenoxazone ring between adjacent base pairs of the double helix¹⁻⁴. There is a general requirement for a guanine base at the actinomycin D binding site on DNA, as illustrated by the observation that actinomycin D does not intercalate into double-stranded (ds) poly(dA-dT) poly(dA-dT) (refs 1-4). Daunorubicin, adriamycin (Fig. 1b) and ethidium bromide (Fig. 1c) also intercalate into dsDNA, but in contrast to actinomycin D, these drugs do not show any requirement for a particular base at the intercalation site^{5,6}. Both actinomycin D and ethidium bromide do, however, show definite preferences for binding to certain sequences of nucleic acid bases at the intercalation site⁷⁻¹¹. In this report we show that daunorubicin and adriamycin facilitate strong binding of actinomycin D to poly(dA-dT) poly(dA-dT) and we discuss the implications of this new observation.

Circular dichroism (CD) spectroscopy is a particularly sensitive technique for monitoring the formation of a complex between drugs and nucleic acids. Both daunorubicin and actinomycin D are optically active and give rise to circular dichroism spectra which undergo characteristic changes when these drugs bind to DNA, synthetic polynucleotides, or oligonucleotides (refs 6, 12-19 and our unpublished observations). The intercalated complex of actinomycin D with DNA is characterised by a large negative band in the 440-490-nm region, whereas actinomycin D in dilute aqueous solution or in solution with poly(dA-dT) poly(dA-dT) has only a negligible band in this region (Fig. 2). Daunorubicin and adriamycin exhibit a broad weak positive CD band in the 440-480-nm region,

which is intensified following binding to DNA or poly(dA-dT) poly(dA-dT) (Fig. 2). The circular dichroism spectra of poly(dA-dT) poly(dA-dT) with daunorubicin or actinomycin D, and of an equimolar mixture of the two drugs with poly(dA-dT) poly(dA-dT) are also shown in Fig. 2. The appearance of the strong negative band in the 450-490-nm region of the spectrum of the equimolar mixture of the two drugs with poly(dA-dT) poly(dA-dT) results from the simultaneous binding of these two drugs to poly(dA-dT) poly(dA-dT). The intensification of the 380-nm band of actinomycin D when it binds to poly(dA-dT) (which is readily apparent in the difference spectrum shown in Fig. 2) also indicates that daunorubicin cooperatively facilitates the binding of actinomycin D to poly(dA-dT) poly(dA-dT). We believe that this is the first example of co-operative binding between two intercalating drugs. Ethidium bromide intercalates into poly(dA-dT) poly(dA-dT), but circular dichroism spectra indicate that ethidium bromide does not facilitate the binding of actinomycin D to poly(dA-dT) poly(dA-dT), nor does the addition of actinomycin D significantly influence the binding of ethidium bromide to this polynucleotide (T.R.K., M.A.Y. & R. V. Kastrup, in preparation). In related experiments, we have used ³H-actinomycin D in equilibrium dialysis experiments, from which we estimate that the association constant for the binding of actinomycin D to poly(dA-dT) poly(dA-dT) in the presence of daunorubicin is $\sim 4 \times 10^5 \text{ M}^{-1}$ (T.R.K., W. E. Moehle & K. G. Rao, in preparation).

These results raise several important questions. First, how does daunorubicin (or adriamycin) enhance the binding of actinomycin D to poly(dA-dT) poly(dA-dT)? Intercalation of these drugs unwinds the DNA helix¹⁻⁶ and it is likely that the distortions induced in the double helix may be transmitted over several base pairs. The amino sugars of daunorubicin and adriamycin, and the cyclic pentapeptides of actinomycin D become located along the grooves of the double helix when the planar portions of the drugs (Fig. 1) intercalate between the base pairs. The

Fig. 1 The chemical structures of *a*, actinomycin D; *c*, ethidium bromide; and *b*, daunorubicin (also frequently called daunomycin) and adriamycin.



R = H Daunorubicin

R = OH Adriamycin

changes in the double helical geometry of poly(dA-dT)·poly(dA-dT) which accompany daunorubicin binding are presumably responsible for the facilitation of actinomycin D binding to an adjacent region of poly(dA-dT)·poly(dA-dT).

Thus the present results show that when daunorubicin binds to poly(dA-dT)·poly(dA-dT) a change occurs in the conformation of the polynucleotide at an adjacent region of

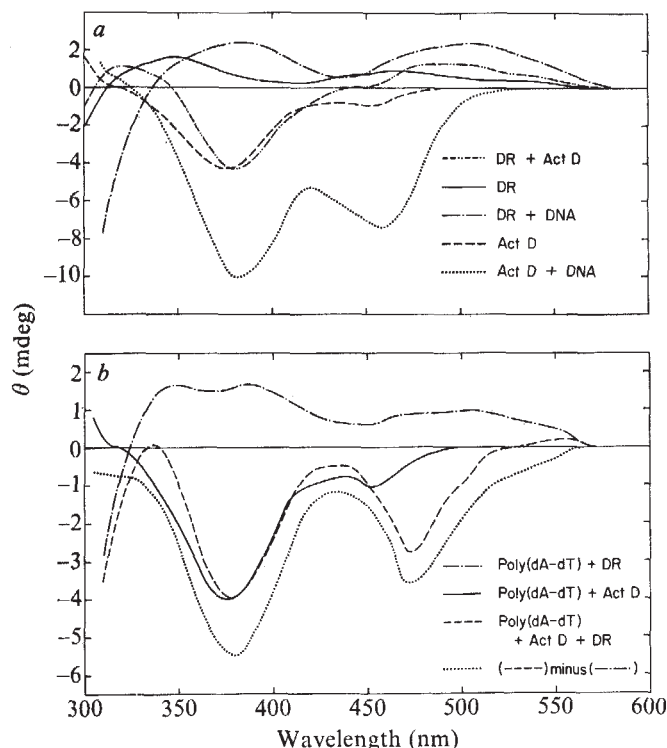


Fig. 2 Circular dichroism spectra of solutions of: *a*, 8.5×10^{-6} M daunorubicin (DR) and actinomycin D (Act D) alone and in the presence of 8.5×10^{-5} M DNA; *b*, 8.5×10^{-6} M daunorubicin plus 8.5×10^{-5} M poly(dA-dT)·poly(dA-dT); 8.5×10^{-6} M actinomycin D plus 8.5×10^{-5} M poly(dA-dT)·poly(dA-dT); 8.5×10^{-6} M actinomycin D plus 8.5×10^{-5} M poly(dA-dT)·poly(dA-dT) + DR + Act D spectrum from the poly(dA-dT)·poly(dA-dT) + DR + Act D spectrum which, to a first approximation, is an estimate of the circular dichroism spectrum of actinomycin D when bound to poly(dA-dT)·poly(dA-dT). All spectra were recorded on a Jasco J-40 circular dichroism instrument in a 4-cm path length cell at 20 °C. All solutions contained 10 mM potassium phosphate buffer, pH 7.0. Essentially similar results were obtained at lower drug-to-phosphate ratios, as well as in the presence of 0.1 M NaCl. The spectra in which adriamycin were used in place of daunorubicin gave qualitatively similar results. 7-Amino-actinomycin D has also been used in place of actinomycin D with qualitatively similar results. The circular dichroism spectra in which ethidium bromide is used in combination with actinomycin D and poly(dA-dT)·poly(dA-dT) do not show the appearance of the large negative band in the 440–480-nm region. The circular dichroism spectrum (*a*) of a daunorubicin and actinomycin D solution (8.5×10^{-6} M in each drug) is not equal to the sum of the circular dichroism spectra of the individual drugs (data not included) at all wavelengths, which shows that a small fraction of the daunorubicin and actinomycin D molecules form a heterodrug complex. These small effects do not influence the present results, however, or the interpretation of the data presented here because >90% of the drugs are bound to poly(dA-dT)·poly(dA-dT) when the polynucleotide is present, and thus the concentrations of the free drugs are $<1 \times 10^{-6}$ M in the presence of the polynucleotides, which essentially eliminates the formation of drug–drug aggregates. We have also studied the binding of actinomycin D and daunorubicin to poly(dA), poly(dT), and a poly(dA)·poly(dT) duplex, where we observed the synergistic binding of the two drugs only in the presence of the ds polynucleotide, poly(dA)·poly(dT). No cooperative binding of the drugs was observed when the ribopolynucleotide poly(A-U)·poly(A-U) was used, which is not surprising because actinomycin D does not bind to polyribonucleotides^{1–4} and daunorubicin has been previously reported to bind only weakly to polyribonucleotides⁶.

the double helix which results in an increase in the stability of the actinomycin D–poly(dA-dT)·poly(dA-dT) complex. This transmission of the distortions of the nucleic acid conformation along the double helix may also be an important component in the selective recognition of nucleic acid sequences (as for example, in promoter–operator complexes), and is related to the transmission of thermal stability from the G-C region to the A-T region which has been observed in block oligonucleotides such as d(C₁₅A₁₅)-d(T₁₅G₁₅) (refs 20–22).

Second, do daunorubicin and actinomycin D (or adriamycin and actinomycin D) both intercalate into poly(dA-dT)·poly(dA-dT)? The shape of the circular dichroism spectra in Fig. 2 suggests that both chromophores are intercalated, but nuclear magnetic resonance experiments on complexes of these drugs with oligonucleotides are required to verify the geometry of the complexes^{8,23,24}.

Third, will these drugs bind cooperatively to DNA *in vivo*, and more important, will they exhibit a synergistic physiological activity if used in combination? *In vivo*, the primary mode of action of the intercalating drugs has been shown to be interference with the nucleic acid polymerases (see refs 25 and 26). In separate experiments (A. H. McHale, S. S. Holcomb, R. Josephson & T.R.K., in preparation) we have observed that actinomycin D does not inhibit the activity of *E. coli* DNA polymerase when poly(dA-dT)·poly(dA-dT) is used as a template, whereas daunorubicin and adriamycin give rise to the anticipated dose–response curves for the inhibition of this enzyme as a function of the drug concentration^{1–4, 6, 25, 26}. The presence of actinomycin D does, however, affect the daunorubicin inhibition of poly(dA-dT)·poly(dA-dT) synthesis, which also illustrates the synergistic interaction of the two drugs when both are bound to poly(dA-dT)·poly(dA-dT). We have been unable to find reports of clinical studies involving the combined use of either daunorubicin or adriamycin with actinomycin D either by themselves or with other drugs. The results reported here suggest that combinations of these drugs should be explored clinically.

We thank R. V. Kastrup and K. G. Rao for assistance with parts of this research. This work was supported by research grants and a Research Career Development Award (to T.R.K.) from the National Cancer Institute. T.R.K. is an Alfred P. Sloan Fellow.

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reviews

Imaginative immunology

Maria de Sousa

B and T Cells in Immune Recognition. Edited by F. Loor and G. E. Roelants. Pp. xviii+504. (Wiley-Interscience: New York and London, 1977.) £18.50; \$37.50.

A RATHER unimaginative title for a rare, imaginative immunology book.

The editors, Francis Loor and Georges Roelants, members of the staff of the Basel Institute of Immunology for long enough to reflect its eclectic atmosphere in their choices, bring together twenty chapters that leave out little that is relevant to those concerned with the way cellular immunology is going.

No complement, gut immunology or parasite diseases. Otherwise, those interested in autoimmunity, immunodeficiency, lymphoproliferative malignancy, HLA-associated disease, cell-mediated cytotoxicity, the major histocompatibility complex (MHC) its role in lymphocyte activation and its relevance to transplantation in man, will find competent chapters by Fye, Moutsopoulos and Talal (autoimmunity), Cooper and Seligman (immunodeficiency and lymphoid malignancy), Ryder and Svegaard (HLA-associated disease), Dausset and Fradelizi (MLR genes and transplantation), Cerottini and Brunner (cell-mediated cytotoxicity) and Bluestein (MHC and lymphocyte activation).

Those wondering whether immunology still has anything to do with the understanding and therapy of cancer will find a concise review of the current state of the clinical and experimental art by Mitchison.

But there is also information for the developmental immunologist. From the wider biological world of LeDouarin, who candidly admits that "the actual immunological function of the thymus is well documented only for Aves and Mammals; only little information has been produced concerning the thymus equivalent organs found in other vertebrates". From the ontogeny perspective of Owen who concludes his chapter on "Ontogenesis of lymphocytes" with the remark that "many basic questions of immunology are essentially problems of lymphocyte differentiation", a remark that gains reality as one wanders through Beverley's chapter on "lymphocyte heterogeneity".

The role of thymic hormones is reviewed by Trainin, Small and Kook. But should one forget that immunology is only T or B cells, an excellent chapter by Roelants reminds us of "the regulatory role of macrophages in immune recognition". This is not the only healthy reminder of the variety of present day immunology.

Lymphocytes "go round", a frequently forgotten event competently reviewed by Sprent in a chapter on "migration and lifespan of lymphocytes"; the chapter by Schreier and Nordin entitled "An evaluation of the immune response *in vitro*" is certain to become a classical chapter of twentieth century immunological literature. This is the only chapter in the book from which the editors requested original data (we are told in the preface). Their intention was to illustrate how cautious one must be with *in vitro* experiments on the immune response. In it, one learns how varying rocking, feeding and adding mercaptoethanol to cell cultures placed in different dishes, leads five different investigators to being equally right in their five different conclusions.

Humour is not restricted to this latter chapter. Parkhouse and Abney's chapter on "Biochemical approaches to receptors for antigen on B and T

lymphocytes" has both humour and sufficient facts and arguments to help readers find their way through an area of much controversy, separately reviewed by Goodman in the chapter on "The specificity repertoire and antigen receptors of T and B lymphocytes".

It is the reviewer's own bias and interest to find evidence of roots of future in present day texts, that leads me to conclude by stressing the importance of the four most enlightening chapters: Loor's on "Structure and dynamics of the lymphocyte surface", Klaus' on "B cell maturation", Nabholz and Miggiano's on "The biological significance of the MLR", and Bretscher's closing chapter integrating T and B lymphocytes in immune activation.

Some minor printing errors: One wonders, for instance if Avrion Mitchison (p 338) did mean to say that "modern immunology starts with Foley and Prehn and Main", knowing with all due respect, who else (including himself) was around at the time.

A rare and imaginative immunology book indeed. □

Maria de Sousa is Head of the Cell Ecology Laboratory at the Sloan-Kettering Institute for Cancer Research, New York.

Quark binding

Quark Confinement and Field Theory. Edited by D. R. Stump and D. H. Wein-garten. Pp. 253. (Wiley: London, New York and Tokyo, 1977.) £18.70; \$31.70.

THIS book offers a collection of papers presented at the workshop on quark binding held at the University of Rochester in June, 1976. The papers represent a cross section of current work on quark confinement and field theory.

Various approaches to the study of collective phenomena, and classical solutions to Yang-Mills field equations are found in papers by Y. Nambu, T. Eguchi, K. Huang and D. Stump, and F. Wilczek. In an attempt to understand a different class of non-perturbative phenomena, N. Snyderman and G. Guralnik discuss dynamical sym-metry breaking.

In two closely related papers, E. Poggio and J. Carrazzone give summaries of their analyses of high orders in perturbation theory for Yang-Mills theories. The stumbling block of this method is, of course, a lack of knowledge about the infrared behaviour of the effective quark-gluon coupling strength. In another paper, P. Olesen offers a description of some aspects of this behaviour using a clever application of the renormalisation group.

Theoretical descriptions of particles produced in e^+e^- annihilation are offered in three papers. V. Matveev presents a phenomenological hydro-dynamic model of collective resonances, a model which he suggests may have applications in the description of e^+e^- production of resonances. A more conventional model is used to describe charmonium by T. Yan. Yan presents

an excellent analysis using an effective field theory with a linear potential. A phenomenological model which attempts to describe charmed particle decays is presented by V. Mathur.

Each of the remaining three papers deals with areas with little connection to other work presented at the conference. In a discussion of covariance problems in two dimensions, C. Hagen questions the validity of light cone field theory. Deviations from scaling are analysed by H. Politzer, using the renormalisation group and the Wilson operator product expansion. An unconventional model involving massive gluons and unconfined colour is presented by R. N. Mohapatra as a possible explanation of high γ anomalies and dimuon events. (It should be noted

that recent experimental work, not yet made public at the time of publication of this collection, offers no evidence for the phenomena this paper attempts to explain.)

This book contains some excellent reports on theoretical work on the quark binding problem and field theory, and would be appropriate for the shelves of physics libraries. For those who are actively working in these areas, however, purchase of this book seems an expensive way to acquire papers which are readily available either as reprints or as published articles.

Larry McLerran

Larry McLerran is a research associate in the Laboratory for Nuclear Science, Massachusetts Institute of Technology, Cambridge, Massachusetts.

Fungal symbiosis

The Biology of Symbiotic Fungi. By Roderic Cooke. Pp. xi+282. (Wiley: London and New York, 1977.) \$21; £10.75.

THE word *Symbiotic* in the title of this book is used in a much broader sense than most botanists have used it in recent years. Instead, the author adopts the older usage of de Bary, recently introduced again by D. H. Lewis, using the term symbiosis to include any association between two organisms which involves long-term intimate contact. This includes, therefore, both antagonistic (parasitic) relationships as well as the mutualistic relationships most commonly considered by botanists to be symbiosis. The author recognises that some relationships exist which cannot with certainty be classified as antagonistic or mutualistic, and these he calls neutral. Neutral symbioses are particularly important in associations of fungi with animals.

Symbiotic relationships between fungi and animals, fungi and higher plants, fungi and algae (lichens) and between fungi and other fungi, are all considered. As explained above, these are classified as antagonistic, neutral or mutualistic relationships; further, the fungi are divided on nutritional grounds, again following D. H. Lewis, into necrotrophic and biotrophic species. In this connection it may be mentioned that the author recognises—and this will be welcomed by many plant pathologists—that quite a number of plant-pathogenic fungi are not easily characterised as necrotrophs or biotrophs; some seem to start their association with their host plant as

biotrophs, finishing up as necrotrophs, and these are classified in this book as a distinct group, hemibiotrophs.

A very wide range of possible associations are thus considered; examples are discussed in each category with particular reference to nutritional and other physiological relationships between the two symbiotic associates. Five chapters are devoted to symbioses with animals and six chapters to symbioses between fungi and plants, including the lichen symbiosis. Since much more is known about fungus-plant symbioses than fungus-animal symbioses, one might expect that the inevitably more 'concentrated' material which appears in the chapters dealing with plants would lead to a degree of imbalance. This is not the case; the book gives a strong impression of unity, and this is all the more valuable in that it covers a range of topics which have never before been brought systematically together.

Indeed, it is striking how similar, in many respects, the fungi involved in biotrophic associations with animals are to those similarly involved with plants. To take one example, it seems to be very common among biotrophs in both situations that they are difficult to culture axenically. This characteristic may become less noticeable as cultural techniques improve, but at present it is quite striking.

This is undoubtedly a valuable book covering a wide field. It will be of interest not only to mycologists, but also to all botanists and zoologists interested in fungal symbiosis. Not many students will be able to afford it—let's hope that it is widely bought by departmental libraries. **P. W. Brian**

P. W. Brian has just retired as Professor of Botany at the University of Cambridge, UK.

Introducing cryobiochemistry

Cryobiochemistry: An Introduction. By Pierre Douzou. Pp. x+286. (Academic: London and New York, 1977.) £12.60; \$24.65.

PROFESSOR DOUZOU'S book on the theory and practice of low temperature biochemistry appears at a time of increasing interest in the technique, and when the first reports of it being used at its full power are beginning to appear. The aim of the technique is no less than the thermal resolution of the individual steps in biochemical processes, especially enzyme reactions, and the stabilisation of intermediates for timescales sufficient for their detailed examination by the more powerful analytical techniques. This is an exciting prospect for the molecular biochemists, and this book is doubly welcome, because not only has Professor Douzou, as the major pioneer of the field, given us the gift of his ten years of experience and development of the subject, but he has also presented it in a well written and clearly organised text.

The major experimental problem of ensuring that even in solutions of mixed solvents at temperatures down to -100°C the proteins are both structurally intact and functionally, potentially competent, is dealt with at length. The book also provides invaluable guidance in choosing solvent systems, the pH, dielectric constant and viscosity at subzero temperatures of which are closely matched with those of the normal room temperature environment. In this respect, the book fulfills the function of a laboratory handbook on low temperature procedures, with many specific examples, drawings of special apparatus and tables of physical properties of mixed solvent systems. At the same time the book reveals its origins as a lecture course, in beginning each section at the most elementary level and taking the reader step by step, from the properties of mixed solvents, through their effects on enzyme activity, to the study of biological molecules at subzero temperatures.

This is a first-class introduction in both the theoretical and practical sense to what is bound to become an increasingly important technique in biochemistry.

C. C. F. Blake

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Hartree-Fock method

The Hartree-Fock Method for Atoms: A Numerical Approach. By C. Froese Fischer. Pp. 308. (Wiley Interscience: London and New York, 1977.) £17.20; \$29.

THE Hartree-Fock (HF) method means different things to different people. The one point in common is that a wave function Φ for a many-particle system is constructed from anti-symmetrised products of one-particle functions ϕ , and that the latter are fully optimised. In many-body theory, as used in nuclear and solid-state calculations, Φ is taken to be a single determinant.

In applications of HF theory to non-relativistic atomic structure calculations, which is the subject of this book, the condition is invariably imposed that Φ must be an eigen function of total spin and total orbital angular momentum operators. In all but the simplest cases, this condition implies that Φ must be a linear combination of several determinants. The HF approximation for atoms is sometimes taken to imply the restriction that Φ is constructed

from functions ϕ , all belonging to the same configuration (although the exact meaning of this statement is not always clear, as is illustrated by Froese Fischer's discussion of the $1s2s^1S$ state). In 1939, Hartree, Hartree and Swirles showed that this restriction need not be made. They introduced the multi-configuration method (MCHF), which has been used extensively in recent years, and provides one of the most powerful known methods for calculating accurate atomic wave functions. A great deal of skill has been devoted to the development of the numerical and computational techniques required for this work, and they are clearly described in this book, which puts particular emphasis on methods using numerical integration of the equations (as opposed to those using expansions in terms of basis functions).

The book gives a lucid account of the foundations of atomic HF theory, a detailed account for the specialist of modern MCHF work, and concludes with a useful discussion of available computer programs for atomic structure calculations.

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Dielectric relaxation

Dielectric Spectroscopy of Polymers. By Peter Hedvig. Pp. 430. (Adam Hilger: Bristol; Akademiai Kiado: Budapest, 1977.) £17.50.

In the past decade, an attempt has been made in many laboratories to unify the study of the dielectric, mechanical and nuclear magnetic resonance (NMR) relaxations in solid polymers, into a coherent spectroscopy. The underlying desire in this attempt was to put forward an interpretation on the molecular level for the observed relaxations. Although such a unified approach has not yet been achieved, Peter Hedvig's book is an excellent up-to-date review on efforts towards this aim.

This first two chapters deal in a concise and precise way with the principles of dielectric spectroscopy and structural transitions and molecular mobilities in solid polymers. Transitions in the glassy state involving local molecular motion are discussed using the potential barrier theory. Cryogenic relaxation phenomena are presented together with the molecular level interpretation. Transitions in the crystalline phase involving defects and orientation effects are discussed, well illustrated and supported by an extensive list of references.

These are followed by three chapters

which present both the experimental techniques and results of dielectric, mechanical and NMR spectroscopies together with dilatometry and differential thermal analysis. The depolarisation technique and the new time domain method (Fourier method) are presented in detail. Dielectric spectra of both pure polymers and polymer compounds are examined both from the chemical structural point of view as well as from the point of view of 'physical' phenomena such as plasticisers and filler effects.

In the last two chapters the study of crosslinking and ageing processes by dielectric spectroscopy is presented. These chapters are unique and original in the literature, and are of special interest to those involved in the technological aspects of dielectric spectroscopy of solid polymers.

In the Appendix the relaxation maps of 31 polymers are presented. This collection is of value to the experimentalist working in dielectric and mechanical spectroscopy fields.

All in all, the book is informative, well balanced, well illustrated and has few errors. It is strongly recommended both for the experimentalist in the field of dielectric relaxation as well as for the advanced student of that domain.

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Metabolic yearbook

The Year in Metabolism, 1975-1976. Edited by Norbert Freinkel. Pp. 353. (Plenum Medical: New York and London, 1977.) \$27.

THIS is the first of a new series of annual publications in which the previous year's developments in selected broad areas of metabolism related to human disease are reviewed. The following topics were chosen: hormone receptors, cyclic nucleotides and control of cell function; diabetes mellitus; glucagon; body fuel metabolism; obesity; lipid and protein metabolism; amino and mono amino organic acids; purine and pyrimidine metabolism; vitamins and minerals; urinary stones; the metabolic aspects of ethanol.

The topics chosen are appropriate because there is updating to be done in each case, and in some cases the authors have had to be selective within their chosen field; for example, chapter 9, "What's New—Vitamins and Minerals", deals only with vitamins C and D, although the author indicates that there may be changes in the future.

I found little of substance to criticise in this book but I am left in some doubt as to its most appropriate target. The honours student in biochemistry will find it much too clinically orientated, and the undergraduate medical student as well as almost all postgraduate medical students will find it much too detailed in a few narrow spheres. It is, in my opinion, a book for specialist metabolic physicians and medical research workers, as well as others who wish to read an up-to-date account of one of the topics covered. It will be invaluable for them.

The real test of this publication's success will be the extent to which the impetus which has brought it into being can be maintained. This implies that each chapter should be re-written for each new edition. If this can be done, it may well provide a needed intermediate between the very condensed but comprehensive publications of the *Annual Reviews* type, and several very good, well-edited and up-to-date large textbooks on the subject, with all of which it will be in competition.

R. W. E. Watts

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Recent scientific and technical books

Mathematics

- CONTI, R. Linear Differential Equations and Control. (Istituto Nazionale di Alta Matematica. Istituzioni Mathematicae, Vol. 1.) Pp.174. (London and New York: Academic Press, 1976.) £6; \$11.75.
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obituary

Wernher von Braun

ONLY occasionally may it be claimed that a single individual has exerted a major influence on a global scale during his lifetime. For von Braun, who died in America on 16 June 1977, the claim may be justified since his work was of critical importance in war and peace throughout the turbulent years of the middle decades of this century. There was, too, a uniqueness about von Braun—that he exerted these influences in two nations divided for half of his life by two World Wars. For although his later triumphs belonged to America, who claimed his citizenship for the last 20 years of his life, he was a German by birth who created a devastating weapon of war in support of Hitler's regime.

Wernher von Braun was born in Wirsitz, Germany on 23 March 1912, the second of the three sons of Baron Magnus von Braun and Baroness Emmy von Braun (née von Quistorp). The ancestral family estates and his early environment might well have led Wernher to a traditional cultural life, but for the circumstance that his mother was an exceptional person. The Baroness was an amateur astronomer who presented Wernher with a telescope as his confirmation gift. We have von Braun's own authority for the statement that it was this event and his early reading of an article in an astronomical magazine describing an imaginary trip to the Moon which fired his determination to join the Verein für Raumschiffahrt (VfR—Society for Space Travel) in 1929. He was then a student at the Berlin Institute of Technology and the VfR, with Hermann Oberth as President, although founded only two years previously, was a thriving concern with over 500 members.

This VfR began the real German experimental work on rocket flight from the *Raketenflugplatz* at Reinickendorf on the outskirts of Berlin. Von Braun participated in the establishment of this 'rocket flying field' from which in 1931 the VfR claimed to have launched a hundred liquid-fuel rockets and made three hundred static firing tests. Altitudes of a mile were achieved but in spite of these successes the VfR almost immediately suffered from the economic depression in Germany. Their difficulties were enhanced by the increasing objections

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Von Braun, arm fractured in a car accident, surrenders to the US Army in 1945. With him, L to R: Maj-Gen Walther Dornberger (Peenemünde Commander) and scientists Lt-Col Herbert Axter and Hans Lindenberg.

of the Berlin police to rocket flights within the city limits.

Ironically it was this rapid decay of the VfR which determined von Braun's future, for, in its desperation the group sought support from the German army. In the summer of 1932 a demonstration of a *Repulsor* (the liquid-fuel rocket of the successful 1931 tests) was given at the army proving grounds at Kummersdorf. This demonstration did not save the VfR—by 1933 it was bankrupt and was finally extinguished by Hitler and the Gestapo—but the army had been greatly impressed by von Braun and invited him to do the experimental work for his doctor's thesis (he graduated in 1932) on rocket combustion phenomena at Kummersdorf.

The army's rocket work was under Captain (later General) Walter Dornberger and his acquisition of von Braun was to prove an event of major significance. Two years later his rockets were reaching an altitude of 6,500 ft, and in 1937 the army rocket group of 80 personnel with von Braun as technical director moved to Peenemünde on the Baltic coast. There, under pressure

from the army to develop a missile with a range of 150 to 200 miles and capable of carrying a one ton warhead, von Braun and his team created modern rocket technology. At last in October 1942 when a test rocket reached an altitude of 50 miles and travelled 120 miles, Hitler ordered top priority for the work.

The attacks against London with the V1 (the buzz bomb) began on 12 June 1944. On 9 September 1944, three days after the Chiefs of Staff reported that all the V1 launching sites had been captured, the world's first ballistic rocket, the V2, fell on London. These attacks continued until 27 March 1945 when all the launching sites had been captured. These V1 and V2 missiles of von Braun had killed 8,938 people and injured 24,504 mainly in the London region.

For von Braun it was an immense achievement—8,000 V1's had been launched against London alone, 1,115 V2's fell on southern England and the Germans claimed another 2,000 had been launched against targets on the continent. The V2 as a ballistic missile was far in advance of anything conceivable elsewhere in the world. Weighing 12 tons at take-off the device was 40 ft long, 5.5 ft in diameter and could carry a one ton warhead 180 to 200 miles. The engine, using a turbo-pump-fed liquid oxygen and alcohol mixture and generating a sea-level thrust of 56,000 pounds worked for a minute after which the trajectory was entirely ballistic. The V2 had an inertial guidance system with two free gyroscopes, levelling pendulums and an integrating gyro-accelerometer. The rocket climbed 60–70 miles and five minutes after lift-off dropped on the target with a speed of 3,500 miles per hour.

Familiarity with contemporary rockets should not be allowed to obscure the monumental nature of von Braun's technological achievement in the development of the V2. It was a weapon which many experts in the Allied countries believed to be impossible to achieve and against which there was no defence. The effect of the V2 on the outcome of the War could well have been decisive if Hitler had not diverted priorities from Peenemünde to the Luftwaffe which curtailed von Braun's

efforts in the period from 1940 until the end of 1942. There were also internal disputes when Himmler attempted to take control of the project—an event which led to von Braun's arrest by the Gestapo and his imprisonment early in 1944 from which he was released after two weeks only by the personal intervention of Hitler.

The second phase of von Braun's career in America culminating in the development of the Saturn rockets which launched men to the moon has received great publicity and is far better documented than the remarkable episodes in 1945 surrounding his escape from Peenemünde and his surrender to the American army. In January 1945 when it was clear to him that Hitler's Reich was about to collapse, he met secretly with his top staff members to decide whether to obey the directive to stand firm at Peenemünde and fall into the hands of the advancing Russians or escape to the south and surrender to the Western Allies. The almost unanimous decision to escape was assisted by an enormous bluff. 5,000 employees and their families together with vast amounts of literature and equipment set out from Peenemünde in February 1945 in railway trains, trucks and cars blazoning prominent signs indicating a purely mythical "Project for special disposition." Carrying one of several contradictory orders implying that rocket research was to continue in the Harz mountains, the von Braun convoy forced its way through road blocks, the SS and Gestapo and eventually reached Bleicherode in the Harz mountains. 12,000 tons of equipment shipped from Peenemünde and earmarked to follow von Braun to the Harz mountains was eventually captured by the Russians in Lubeck.

At Bleicherode von Braun found himself faced with the SS General Kammler who had earlier tried to take over Peenemünde, but Kammler thought he could bargain better with the approaching Allies if he used von Braun and his team as hostages and he placed them in a prison camp at Oberammergau. Eventually von Braun managed to disperse his group into the surrounding mountains and it was from that region of the Bavarian Alps that he surrendered to the Americans early in May 1945. They had acquired one of the great technical prizes in history for, apart from the Peenemünde team, the huge underground V2 assembly plant at Niedersachswerfen was captured intact.

When he arrived at Bleicherode, von Braun hid 14 tons of documents about the Peenemünde developments in a tunnel near Dörten at the northern edge of the Harz mountains. These and large amounts of the V2 material

Popperfoto

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3 February 1958. Holding a model of the successful Explorer satellite launch vehicle, L to R: William Pickering, Director of the Jet Propulsion Laboratory; James Van Allen; von Braun.

(enough for about 100 rockets) were removed from the region only days before the Russian army moved in. Sixteen Liberty ships transported the cargo from Antwerp to New Orleans and within a year the Americans were gaining invaluable experience and commencing their upper atmosphere researches by launching the reassembled rockets from White Sands, New Mexico.

These complex and urgent movements were under the command of the US General Holger N. Toftoy, Chief of the Ordnance Technical Intelligence team in Paris. It was Toftoy who recommended that von Braun and his technical staff should be brought to the United States. On 23 July he was ordered to arrange the move but only for 100 of the staff and in early August 1945 he met von Braun at Witzenhausen to offer him a one year contract in the US under Ordnance Corps custody. Before finally leaving for the US von Braun spent two weeks in England for meetings with Sir Alwyn Crow and other members of the Ministry of Supply who had been involved in the attempts to develop British rockets during the war.

His American life began on 29 September 1945 when, with seven other members of the team, he was met at Fort Banks in Boston harbour by Col. James P. Hamill who was to be his C.O. for many years. Soon, von Braun was at Fort Bliss in El Paso, Texas. Gradually his team arrived and by February 1946 they were complete and at work on assembly and testing of the converted V2's. The first launching from White Sands, New Mexico was on 16 April 1946 and the launchings of the captured V2 rockets continued for six

years during which time the U.S. Army continued to move forward from the V2 technology under von Braun's guidance.

In 1947 von Braun was given permission to return to Bavaria to marry his 18 year old second cousin Maria Louise von Quistorp, and there are two daughters and a son of the marriage.

In October 1949 the Secretary of the Army gave formal approval for the transfer of the rocket group to the Redstone Arsenal in Huntsville, Alabama. The move in 1950 involved 500 military personnel, several hundred U.S. civilians and the technical core of von Braun and 130 members of his original Peenemünde staff. Hamill was in charge, with von Braun as director of the Guided Missile Development Division.

These were critical years for the US missile developments—spurred by the needs of the Korean War the Redstone missile was developed. First fired from Cape Canaveral in August 1953 it was the first missile to be in operational use in the US Army and was soon to play an unforeseen and dramatic part in the space research.

At that time intelligence sources found evidence that the USSR was in an advanced stage of development of an intercontinental ballistic missile (ICBM). All three US armed services were thereby spurred to vigorous development of an ICBM. The creation of the Army Ballistic Missile Agency in Huntsville under General Medaris was centered around von Braun as technical director. The Redstone rocket was upgraded and two upper stages were added to form the vehicle known as Jupiter C. The first stage

was a Redstone missile lengthened to hold more propellant and the engine was modified to burn a new fuel—hydrazine, a mixture of unsymmetrical dimethylhydrazine and diethylene triamine. The second stage was a cluster of eleven solid-propellant rockets and the third stage fitting inside the ring of the second stage comprised three solid fuel rockets. Von Braun stabilised the system by spinning it rapidly during flight and on its first flight from Cape Canaveral in September 1956 this remarkable vehicle reached an altitude of 682 miles and travelled 3,400 miles.

Ironically and almost immediately the Army was ordered to concentrate on short range missiles while the control of the ICBM programme passed to the Air Force. External events, however, once more determined a different destiny for von Braun.

In 1954 he showed that it would be possible to launch an earth satellite using the Redstone rocket assemblies as in the Jupiter missile. His scheme, under the code name Project Orbiter, was considered by an advisory group under Assistant Secretary of Defence Donald A. Quarles who outvoted the idea by 7 to 2 at a meeting on 9 September 1955, in favour of Project Vanguard, an alternative proposal from the Naval Research Laboratory. The official record states that the decision was based on technical recommendations of the advisory group. More probably the phrase covers the persuasion of President Eisenhower that the military association and the constraints of the secrecy classification on the Jupiter C rocket would be harmful to the peaceful intent of the satellite project. In the event Vanguard, based on sounding rocket technology and aside from the main stream development of the U.S. military ballistic rocket, inadequately financed and with no appropriate priority, was an almost total disaster.

In the turmoil created by the success of Sputnik One on 4 October 1957 von Braun again pressed his case from Huntsville that he should be allowed to use the army's Jupiter missile to launch a satellite. Three weeks after the launching of Sputnik he was given three and a half million dollars with a target date of 30 January 1958. Once more he triumphed—on 31 January the Jupiter rocket placed Explorer 1, the first American satellite in Earth orbit which within a few days discovered the zones of trapped particles around the Earth (the van Allen zones). The decade of intense technological rivalry between the US and USSR following these events would have borne a radically different aspect if von Braun's advice had been followed in 1955.

After these events the Eisenhower administration took immediate action to create a unified US space effort. Following the advice of a committee under James R. Killian steps were taken to create a strong civilian-orientated agency to direct the manned and unmanned exploration of space. The National Aeronautics and Space Act became law on 29 July 1958. Successively this National Aeronautics and Space Administration absorbed the 8,000-strong National Advisory Committee for Aeronautics, the Vanguard and NRL groups, the 2,800 staff of the Jet Propulsion Laboratory of CalTech and then in January 1960, President Eisenhower decreed that the vital army group, with 4,600 personnel under von Braun should be transferred from army to NASA control. On 1 July 1960 the transfer was effected when the President personally visited and dedicated the facility as the George C. Marshall Space Flight Center with von Braun as director.

Four years earlier von Braun had begun studies of rockets which would be far more powerful than the Redstone combinations. He knew that the rockets required for intercontinental missiles would never provide the thrust required to place man into space. In August 1958 he already had approval to develop a multiple Redstone/Jupiter combination with a thrust of 1.5 million pounds, and by the time of his absorption into NASA he had a priority rating for the development of vehicle known as Saturn 1. In October 1961 this 162 ft long carrier rocket weighing a million pounds had its flawless test launching from Cape Canaveral. By January 1964 a 3,700 pound test payload was placed in orbit. Thereafter von Braun progressed majestically and incredibly to the targets of the Apollo programme and to the fulfilment of his vision.

These early Saturn vehicles were mere stages towards the mammoth Saturn 5, the development of which was approved by NASA in January 1962. Standing 364 ft high, Saturn 5 weighed over six million pounds and could send 47 tons of payload to the Moon or place 140 tons in Earth orbit. The landing of Armstrong and Aldrin on the Moon in July 1969 was an operation of extraordinary complexity; an immense collaborative enterprise which von Braun and the Huntsville team made possible by designing this multi-million pound thrust rocket. From assembly to the count-down the monitoring system alone needed 10 miles of tape storing over 2.5 million words.

Von Braun witnessed the fulfilment of a youthful dream in his fifty-eighth year. No longer did it seem technical fiction that he wanted to proceed even

further along this path. He had designs to get man even further into space. First he would have strapped on solid rocket motors to the first stage of Saturn 5, then to get the men to Mars he planned to replace the existing liquid hydrogen-oxygen third stage by a nuclear engine. But this remained a dream; NASA was forced to cut the space programme and within a few years of the climax of Apollo 11, von Braun had the chagrin of witnessing the dismantling of the facilities and the dispersal of the men who had made Saturn a reality.

In 1970 von Braun was made Deputy Associate Administrator for Plans in NASA, an office which he held until his retirement at the age of 60 two years later. He then joined Fairchild Industries as Vice President for Engineering and Development but became ill with cancer and retired finally a few months before his death.

Von Braun was an extraordinary symbol of the age. His vision was of a nature which the establishment looked upon as science fiction but by single-minded persistence he astonished the world by succeeding through the labyrinth of war and peace, first as a German and then as an American citizen. Although von Braun was granted American citizenship in 1955 and rescued the Americans from their despair in the face of the Soviet Sputnik, there were those who could not forget that he laboured for the Nazis. But for von Braun the transformation was complete. He built his rockets to get men away from the earth—his imprisonment during the Peenemünde era resulted from an unwise remark that the V2 would help towards space travel—and he succeeded.

Bernard Lovell

D. W. Holder

At the time of his death on 18 April 1977, Professor Holder had been Head of the Department of Engineering Science at Oxford for close on sixteen years. In that time the laboratory had expanded at both the undergraduate and postgraduate level and much of the planning for this growth stemmed from his own initiatives.

He was educated at Imperial College in the Department of Civil Engineering between 1941 and 1943 and subsequently in the Aeronautics Department under Sir Leonard Baird. After graduating he immediately joined the Ministry of Aircraft Production at the Aircraft and Armament Experimental Establishment at Boscombe Down but soon transferred to the Aero-

dynamics Division at the National Physical Laboratory where he remained until 1961. His promotion from the junior grades to Deputy Chief Scientific Officer in 1957 was rapid and his career at the NPL spanned some of the most dramatic changes seen in aeronautics.

At the end of World War II, little was known about the behaviour of aircraft at transonic and supersonic speeds but the next decade was to change all that and Douglas Holder played an important role from the outset. The first problem was to design and build the wind tunnels which would enable the aerodynamic phenomena to be investigated. The Aerodynamics Division chose to use induced flow tunnels and a series of these ranging from the original pilot model 9 inches by 3 inches in cross section to 36 inches by 14 inches was constructed.

Before 1949 research on flow at Mach numbers close to unity had been impossible because of the reflection of the normal shock from the tunnel wall. Extensive studies of slotted walls were conducted in the UK and the USA and Holder's group at NPL made substantial contributions to this new technique which could eliminate tunnel interference effects. In addition to this work on tunnel design he made, with Gadd and Pearcey, important advances in shock wave boundary layer interaction and aerofoil design so that the division as a whole established an enviable international reputation.

Following closely on this pioneering research on transonic and supersonic aerodynamics Holder next led the development of a series of shock and wind tunnels for the study of flight at hypersonic speeds, i.e. above about Mach 7. Completely new techniques were needed to drive such facilities and Holder, now head of all high speed aerodynamics at NPL, took a major role in planning and designing these devices. The flow durations in shock tubes and shock tunnels range from tens of microseconds to a few milliseconds but Holder was equally at home in this new field. He had very early demonstrated a highly developed skill in wind tunnel design and had the ability, which may be the mark of the true engineer, to make rapid estimates of all the important design parameters for a new facility. Throughout this period he published, as he had always done, widely, and some of his papers are classics of their kind.

In 1961 he was invited to take the Chair of Engineering Science at Oxford to succeed Thom who was then retiring, and became at 37 the fourth holder of that post. The early sixties saw in Oxford, as in the UK as a whole, a rapid expansion of university engineer-

ing departments and under Thom a major development had been initiated. Holder took over this work and guided it through to completion. Although he continued to take a keen interest in fluid dynamics he also saw the importance of maintaining and extending a broad base of knowledge and there are now two new chairs in structures and electricity, largely due to his foresight and persistence.

His long association with aeronautics at the NPL made him an obvious choice as a consultant for industry and government and he was also much in demand in educational establishments. He was elected FRS in 1962 and served on many of its committees as well as its Council between 1969 and 1971. His wide range of activities outside Oxford constantly suggested new lines of research and development and together with Professor R. B. Duthie he established in Oxford a centre for orthopaedic engineering and encouraged work in physiological fluid dynamics.

Outside of Oxford his government advisory work was at the highest level and must remain obscured by confidentiality although he will be remembered for his chairmanship of the enquiry into Precision Approach Radar following the Gatwick disaster in 1969 and later, in 1971, his involvement at ministerial level in the technical decisions on the future of the RB-211 engine.

By nature he was reserved and the extent of his influence in both aeronautics research and engineering education is a testimony more to his patient and persuasive skills than to any justifiable forcefulness in argument. He was an exceptionally able administrator and dealt with what would appear a crippling work load with quiet efficiency. He was always ready to listen to his staff and research students and to encourage them to pursue new lines of investigation and he was generous to a fault to those who were in difficulties, although few around him would be aware of his very personal interventions. In this respect he was particularly effective on the University Staff Committee where he will long be remembered for his patient and skilful administration.

In his views of the part that universities should play in the industrial life of the country he was ahead of his contemporaries and with such wide experience and extensive contacts he often put his finger on promising lines of new work long before they were apparent to others. There are, alas, only a few of his calibre and he will be sadly missed in both university and industrial bodies.

D. L. Schultz

Yves Guitton

PROFESSOR YVES GUITTON died on 6 July 1977 in Perpignan (France) at the age of 46. He leaves a wife and two sons. Head of the Laboratory of Plant Physiology, he was also chairman of the University Group for the Biology of Development and Parasitology and Vice-president of the University of Perpignan.

With a wide-ranging scientific background, involving organic chemistry, nuclear energy research and microbiology, he entered plant physiology and biochemistry because he felt the development of this field to be essential for the future. His doctoral thesis was devoted to the metabolism of arginine during the germination of *Pinus*, and his contribution to the study of amino acids and nitrogenous substances was invaluable to French biochemistry.

In 1966 he was appointed to the newly-created University of Perpignan, addressing himself to a problem which intrigued him: the role of nucleic acids in developmental processes. Arriving alone in his new laboratory, geographically isolated from the scientific community, Professor Guitton nevertheless created within ten years a laboratory equipped for all techniques of molecular biology. The research team which he built up is a leading group in French physiology, collaborating with others both at home and abroad.

His recent contributions concern the metabolism of mRNA during the first hours of seed germination, mRNA stability and development-related changes in chromatin structure and composition. With his collaborators he was the author of around fifty publications in French and international journals.

In addition to his brilliant scientific career he involved himself with equal enthusiasm in his outside interests. An excellent footballer in his youth, he turned his attention to tennis after a serious accident, rapidly reaching a high standard. Many will remember his arrival at scientific conferences complete with tennis racquet, looking for partners among his American and British colleagues. In politics he was a highly active member of the Socialist party, deeply involved in the problems of his city.

For those who knew him Yves Guitton will be remembered not only for his exceptional achievements, but perhaps even more for his enthusiastic approach to all which concerned him and his warmth and friendliness in any company. His premature loss will be deeply regretted by his many friends in the scientific community and in his city. Michel Delseny